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CANADIAN JOURNAL OF AGRICULTURAL SCIENCE

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THE ESTABLISHMENT OF INTRODUCED PARASITES OF THE EUROPEAN SPRUCE SAWFLY (*DIPRION HERCYNIAE* (HTG.) (HYMENOPTERA : DIPRIONIDAE) IN THE MARITIME PROVINCES[1]

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[Received for publication December 4, 1952]

ABSTRACT

Large numbers of parasites were introduced against the European spruce sawfly, *Diprion hercyniae* (Htg.), in the Maritime Provinces from 1934 to 1949. The majority were liberated during the sawfly outbreak, which ended about 1939. Seven species became established. Their establishment, survival, known distribution, and spread from liberation points are discussed. Two species of cocoon parasites were recovered. Only one of these, *Dahlbominus fuscipennis* (Zett.), was taken in large numbers, and this appeared to be a density-dependent species. Five species of larval parasites were recovered, including four species of *Exenterus* and a tachinid parasite, *Drino bohemica* Mesn. Two species of *Exenterus* showed rapid establishment at high levels of sawfly population, but these species were recovered infrequently after the end of the sawfly outbreak. *E. vellicatus* Cush. and *D. bohemica* were slow in becoming established, and it was not until the end of the sawfly outbreak that their effectiveness was realized. These are the most persistent of the introduced parasites at low densities of sawfly population, and they promise to be permanent additions to the control complex of the sawfly.

INTRODUCTION

Outbreaks of the European spruce sawfly, *Diprion hercyniae* (Htg.), lasted from 4 to 15 years in Eastern Canada and the recovery of surviving spruce trees dates from about 1940 in most areas (32). During the years of severe outbreak, an extensive program of parasite introduction was carried out by the Division of Entomology, Department of Agriculture, through the Dominion Parasite Laboratory, Belleville, Ontario, and the Imperial Bureau of Entomology. Similar programs were conducted in the United States by the Bureau of Entomology and Plant Quarantine and the State of Maine Forest Service.

Many promising species of parasites were introduced from Europe and Japan (3). The establishment of some species has been reported by Baird (1, 3, 4, 5), Balch (7, 8, 9), Briand (11, 12), and Reeks (31) in Eastern Canada, and by Dowden (15), Dirks (14), and Peirson and Nash (27) in the northeastern states of America. However, very little has been published on the status of the introduced species after the end of the sawfly outbreak.

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[2] Agricultural Research Officer.

This paper summarizes records of parasitism during and following the sawfly outbreak in the Maritime Provinces and two areas in the Province of Quebec. It discusses the establishment of successful species at various levels of sawfly population, their spread from liberation points, and their distribution. Brief accounts of the seasonal development of the most prevalent species are also included, with emphasis on aspects of their behaviour that affect establishment. The species recovered are the cocoon parasites *Dahlbominus fuscipennis* (Zett.) and *Aptesis basizonia* (Grav.), and the larval parasites *Exenterus claripennis* Thom., *E. amictorius* (Panz.), *E. vellicatus* Cush., *E. tricolor* Rmn., and *Drino* (*Prosturmia*) *bohemica* Mesn. It is clear from the rearing of large numbers of sawfly cocoons or larvae that none of these species were present in Eastern Canada prior to the liberations that were initiated in 1933 (30).

Most of the studies were carried out at Fredericton, N.B., and on a plot at Young's Brook, York County, N.B. The establishment of the parasites was investigated on many other plots, particularly those located at Parke Reserve, Kamouraska County, Que., and the upper watershed of the Grand Cascapedia River in the Gaspé Peninsula. The nature of the stands and history of the sawfly outbreak on these plots were described in an earlier paper (32).

METHODS

The most widely used method of sampling for larvae was that described by Balch (6). It consisted of beating the foliage of spruce trees with a 10-foot pole, and collecting the sawfly larvae on a sheet measuring 7 by 9 feet. This procedure was commonly used by the Forest Insect Survey. Several hundred co-operators of the Survey collected the samples at random. These were reared in insectaries at Fredericton or Halifax. The collection and rearing of such samples showed the host population as numbers per tree-sample, and provided data on parasitism and distribution. A similar method was used by those engaged in studies on the sawfly disease (10). The latter differed primarily in the aseptic conditions under which the samples were collected and reared.

Cocoon collections were taken by two methods. One consisted of collecting, at random, fairly large numbers of sound cocoons at a large number of locations. Autumn collections predominated, but some were taken during the spring and summer.

The second method of cocoon sampling was similar to that described by Prebble (28). Permanent plots were established, and on each plot up to 200 square feet of forest floor were examined annually, either as 1-square foot or 4-square foot quadrats. Hence parasitism could be correlated with host density. The results from all the plots are not shown but those selected are representative of many areas in New Brunswick. The presence of parasites in the plot samples was determined principally from rearings for emergence.

In summarizing the parasite recoveries, the percentages of parasitism are based on the "total sound" cocoons, which are those that produce either sawfly or parasite adults. Some of the figures are based on extremely small numbers of cocoons, particularly in recent years. In some years the

sampling of 200 square feet of forest floor, requiring the work of 20 to 30 man-days, produced only one or two sound cocoons. At this population level it was impossible to extend the sampling for results of statistical significance. All percentages of parasitism given for cocoon collections tend to underestimate the effectiveness of the parasites. They exclude the emergents of earlier generations and those parasites that issue from sawfly larvae on the trees. Larval collections provide a more reliable figure on the effectiveness of larval parasites, but even these introduce the error of removing the host before it completes its normal period of exposure to parasite attack.

PARASITE LIBERATIONS

This paper is concerned with the liberations of only those species that were later recovered from field collections. A summary of these (Table 1) is based on the lists recorded in Volumes 16 to 27 of the *Canadian Insect Pest Review*. A more detailed account of the liberations on or near the sawfly population plots is given in Table 2. As information on the taxonomy and the establishment of parasites increased, it became apparent that some of the early colonies contained more than one species. Conse-

TABLE 1.—LIBERATIONS OF INTRODUCED PARASITES THAT BECAME ESTABLISHED ON THE EUROPEAN SPRUCE SAWFLY IN THE MARITIME PROVINCES

Species	Liberations		
	Number	Years	Province
Cocoon parasites:			
Hymenoptera : Ichneumonidae			
A. basizonia	115,400	1933 to 1940	N.B.
Hymenoptera : Eulophidae			
D. fuscipennis	268,635,500	1934 to 1946	N.B.
	15,320,000	1936 to 1945	N.S.
	9,240,000	1942 to 1948	Nfld.
	700,000	1941, 1946	P.E.I.
Larval parasites:			
Hymenoptera : Ichneumonidae			
E. amictorius	7,477	1942 to 1944	N.B.
	2,216	1942 to 1945	N.S.
	4,478	1943 to 1947	Nfld.
	750	1944	P.E.I.
E. claripennis	6,635	1940 to 1943	N.B.
	1,200	1940, 1942	N.S.
	329	1943, 1944	Nfld.
E. tricolor	323	1937 to 1939	N.B.
Exenterus spp., mixed lots of: *claripennis*, *amictorius*, *vellicatus*, or *adspersus*	11,200	1934 to 1941	N.B.
	755	1936 to 1939	N.S.
Diptera : Tachinidae			
Drino spp.	42,100	1934 to 1948	N.B.
	21,100	1936 to 1949	N.S.
	51,800	1943 to 1949	Nfld.
	11,600	1942 to 1947	P.E.I.

TABLE 2.—LIBERATIONS OF PARASITES OF THE EUROPEAN SPRUCE SAWFLY IN RELATION TO SAMPLING UNITS ON THE POPULATION PLOTS. (SEE TABLES 4, 5)

Location of plots	Species	Liberations		Distances in miles between lib. pts. and sampling units
		No. parasites	Years	
Parke Reserve, Que.	*A. basizonia*	52,700	1934–1939	0.3–0.5
	D. fuscipennis	651,700	1934, 1935	0.3–0.5
	Exenterus spp.*	42,000	1934, 1935	0.3–0.5
	E. tricolor	206	1937–1939	0.3–0.5
	E. vellicatus	62	1939	0.3–0.5
	Drino spp.	848	1934–1936	0.3–0.5
	Drino sp. (Japanese)	878	1936, 1937	0.3–0.5
McNamee, N.B.	*A. basizonia*	4,200	1934	0–1.0
	D. fuscipennis	247,800	1937	0–1.0
	Exenterus spp.*	445	1934	0–0.1
	Drino spp.	287	1934	0–0.1
	Drino sp. (Japanese)	600	1937	0–0.1
Dunbar, N.B.	*D. fuscipennis*	40,000	1935	0.1–0.2
	Exenterus spp.*	404	1939	0–0 1
	Drino spp.	276	1939	0–0.1
Canaan River, N.B.	*A. basizonia*	117	1936	0.2–0.3
	D. fuscipennis	100,000	1936	0.2–0.3
	Exenterus spp.*	65	1936	0.2–0.3
	Drino spp.	18	1936	0.2–0.3
Young's Brook, N.B.	*A. basizonia*	22,000	1937	0.1–2.0
	D. fuscipennis	835,000	1936, 1937	0.1–1.0
	Exenterus spp.*	2,393	1936, 1937	0.3–0.4
	E. tricolor	23	1937	0.3–0.4
	Drino spp.	636	1937	0.3–0.4
	Drino sp. (Japanese)	1,559	1937	0.3–0.4
Acadia Station	*A. basizonia*	480	1938	0–0.1
	D. fuscipennis	1,000,000	1938	6.5
	Exenterus spp.*	1,519	1938	0–0.1
	E. tricolor	188	1938	0–0.1

* "*Exenterus* spp." refers to lots that were probably mixed, containing *claripennis*, *amictorius*, or *vellicatus* and excludes *abruptorius* and *tricolor*. Liberations near Parke Reserve plot given to 1939.

quently, the lists of species and numbers liberated are in accordance with the latest revisions in nomenclature.

The distribution maps of Baird (3, 4) show that for the first few years the liberations in the Maritimes were concentrated in central New Brunswick, but after 1937 the parasites were more uniformly distributed in the accessible infested forests of New Brunswick and Nova Scotia. The greater concentration of liberations in New Brunswick was due to the earlier and more severe sawfly outbreaks in this Province. Infestations were generally light in the other Maritime Provinces, except for a few areas in northern Nova Scotia and western Newfoundland.

ESTABLISHMENT BY SPECIES

Dahlbominus fuscipennis (Zett)

The life history of this species has been discussed in several papers (16, 24, 29), and is shown diagrammatically in Figure 1 for an average year in New Brunswick.

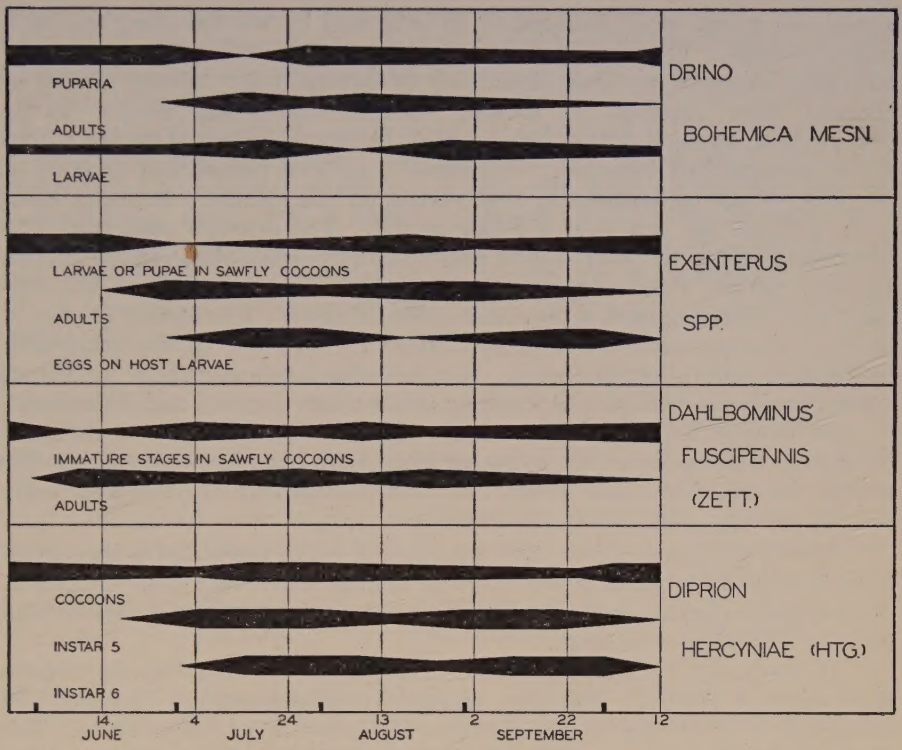

FIGURE 1. Occurrence of stages of introduced parasites and the European spruce sawfly by approximate periods in central New Brunswick.

Some Factors Affecting Establishment

Multiplication.—The success of *D. fuscipennis* is favoured by its ability to multiply rapidly, high fecundity (16, 24), and a preponderance of females (15, 27, 29). Each of these factors may vary with weather or climate.

The number of generations under field conditions was generally three but sometimes two in central New Brunswick. The parasite laid up to 215 eggs in the laboratory, but in the field 70 was the average and 132 the highest. The percentages of female adults reared from parasitized, field-collected cocoons was 81 per cent of 5,508 adults at Young's Brook and 91 per cent at Parke Reserve (29). The biotic potential of the parasite is at least four times greater than that of the sawfly, but it is doubtful if a parasite, laying its full complement of eggs, would attack more than three or four host cocoons under normal conditions.

Biotic Factors.—Morris (22) stated that in areas where sawfly cocoons had been abundant for several years, 40 to 50 per cent were generally destroyed by small mammals. Balch and Bird (10) showed that about 80 per cent of the dormant cocoons in one area were destroyed by small mammals and other causes. Experiments by Morris (23) indicate that some small mammals can distinguish between sound and empty cocoons. Hence most of the cocoons opened by mammals contain living insects. However, they are unable to distinguish cocoons containing the sawfly from those

containing pupae of *D. fuscipennis*, as indicated by the following test with caged animals. The cage and conditions under which the test was carried out were identical to those described by Morris (23). Three classes of cocoons were exposed to attack, cocoons containing sawfly larvae (eonymphs), cocoons containing pupae of *D. fuscipennis*, and cocoons from which sawflies had emerged. The small supply of parasitized cocoons at the time of the experiment in 1946 restricted the numbers to about 10 of each category per day over a 3-day period. The animals used were one specimen each of the short-tailed shrew, *Blarina brevicauda pallida* Smith, the deer mouse, *Peromyscus maniculatus abietorum* (Bangs), and the red-back vole, *Clethrionomys gapperi ochraceus* (Miller). The results (Table 3) indicate that parasitized and unparasitized cocoons are equally acceptable to the three species of mammals. It is probable that under field conditions small mammals cause proportionately equal losses of hosts and parasites.

In Europe, the effectiveness of *D. fuscipennis* is sometimes restricted by hyperparasites (26). No hyperparasites have been found attacking this species in the Maritime Provinces, but *fuscipennis* itself occasionally attacks primary parasites in the sawfly cocoon.

Nature of Humus, Litter, and Moss Layer.—The sawfly spins its cocoon in or immediately beneath the layers of humus, litter, or moss. Adults of *D. fuscipennis* must penetrate barriers that vary in depth and composition in order to find host cocoons.

In one sampling test, the humus and moss layers beneath spruce trees were divided roughly into two classes. One consisted of humus and mosses, principally species of *Calliergon*, *Hylocomium*, or *Polytrichum*. The average depth of the combined layer was about $2\frac{1}{4}$ inches, ranging from 1 to 4 inches. The other class consisted principally of a shallow humus layer and litter, with sparse herbaceous growth. The depth averaged about $1\frac{3}{4}$ inches, and ranged from 1 to $2\frac{1}{2}$ inches. Sample units, of 4 square feet each, were examined from each class at points where colonies (10,000 parasites per colony) were released two years earlier. The mean number of parasitized cocoons per 4-square foot area were 2.2 for 18 samples of the first class, and 1.5 for 44 samples of the second class. The difference was not statistically significant, indicating that the cocoons in the two classe of humus and moss layers were almost equally accessible to the parasite adults.

TABLE 3.—RESULTS OF CAGE EXPERIMENTS TO TEST DIFFERENTIATION BY SMALL MAMMALS IN SELECTING COCOONS OF *Diprion hercyniae* (HTG.), FREDERICTON, N.B.

Species	Type of cocoon	No. opened	No. unopened	Total	Per cent opened
Blarina	*Diprion* living	18	18	36	50.0
	Dahlbominus pupae	20	12	32	62.1
	Diprion emerged	15	21	36	41.5
Peromyscus	*Diprion* living	30	0	30	100.0
	Dahlbominus pupae	29	0	29	100.0
	Diprion emerged	16	15	31	51.3
Clethrionomys	*Diprion* living	20	4	24	83.3
	Dahlbominus pupae	20	5	25	80.0
	Diprion emerged	20	12	32	62.2

Dirks (14) suggested that the degree of parasitism by *D. fuscipennis* probably varies inversely with the amount and depth of moss on the ground. Samples were taken in central New Brunswick to show how far the parasite can penetrate the forest floor. Cocoons were collected from three strata. The upper stratum, depth about $1\frac{1}{2}$ inches, contained 221 cocoons, of which 7.3 per cent were parasitized; the centre stratum, depth about $1\frac{3}{4}$ inches, contained 180 cocoons, of which 8.3 per cent were parasitized; and the lower stratum, depth 2 to 4 inches, contained 235 cocoons, of which 11.5 per cent were parasitized. It is probable, as mentioned by Balch (7), that the accessibility of the cocoons to *D. fuscipennis* is determined more by the temperature of the forest floor than by the degree of mechanical obstruction.

Temperature and Climate.—Morris and Cameron (24) found that cocoons of *Neodiprion sertifer* were attacked by *D. fuscipennis*, regardless of whether they were spun on the tree trunks or on the forest floor. In 1936 a simple test was carried out to aid in determining the "searching zone" of adults in Canada. Small screen cages, each containing 15 cocoons, were distributed near a parasite liberation point. The spacing of the mesh permitted the entry of parasite adults. Eighty-two lots were placed on the ground at distances up to 450 feet from a point where *D. fuscipennis* was released two years earlier. Nineteen lots were located at points on the lower 14 feet of a spruce tree at the immediate point of liberation. The cages were left undisturbed for about two weeks, when the cocoons were analysed, and a fresh supply was added. Slightly over half of the lots on the ground, and 24 per cent of the total cocoons, contained parasites, but none of the cocoons on the stem were attacked. This suggests that the parasite is attracted in some way to the general location of the host cocoons, although Ullyett (34) proposed that the initial step in the discovery of the host is due to chance. A contributing factor to the final step in finding the host is the temperature of the forest floor. The question arises as to whether this temperature falls within the "temperature preferendum" as defined by Wilkes (35).

Wilkes defined the "temperature preferendum" as the temperature zone at which 50 per cent to 80 per cent of the adults come to rest. He found that the average preferred temperature for normal laboratory stock lies between 70.2° F. and 73.0° F., but the preferendum could be lowered by selective breeding. He observed that 55 per cent of the individuals of "wild stock" preferred temperatures below 59° F., or an average of about 50° F. The average maximum ground temperatures (thermometer bulb in moss layer about 2 inches deep) at three stations in the upper Cascapedia Watershed in 1938 for the months of June, July, August, and September were 53° F., 58° F., 58° F., and 48° F., respectively. Corresponding temperatures in 1940 at a point in central New Brunswick were 53.0° F., 60.5° F., 60.5° F., and 56.6° F. At the latter point maximum ground temperatures exceeding 50° F. occurred 95 days during the season, and those exceeding 59° F. occurred 44 days. The maximum ground temperature for the season was 68° F. Therefore, the ground temperatures of central Gaspé and parts of New Brunswick would tend to be lower than the "temperature preferendum" of laboratory stock but possibly within

the range of that of selected stock. As suggested by Wilkes (35), the insect may have undergone a selective process in nature, resulting in a lower preferendum and possibly enabling it to cope with various conditions of climate.

Oviposition by *D. fuscipennis* is generally highest in the more open stands, which tend to have higher ground temperatures. The lowest temperature at which oviposition was observed in the field was 46° F. The rearing of 95 lots of adults in cages in a spruce stand near Fredericton showed that the degree of oviposition increases directly with ground temperature. Each lot consisted of 15 one-day old female adults. They were confined with 15 sawfly cocoons in a 4- by 4- by 1-inch wire screen cage for 24 hours, and the cage was restocked daily with a fresh supply of parasite adults and sawfly cocoons. The cage was located at the bulb of a ground temperature recorder, which was checked daily with thermo-electric equipment. The daily maximum ground temperatures in relation to the average eggs per lot, were as follows: Temperatures 46° F. to 50° F., 4.2 eggs, 15 observations; temperatures 51° F. to 55° F., 26.7 eggs, 25 observations; temperatures 56° F. to 60° F., 67.2 eggs, 28 observations; and temperatures 61° F. to 66° F., 98.5 eggs, 25 observations. Thus the highest oviposition occurred at maximum ground temperatures that were higher than the monthly means (shown above), indicating that temperatures in the moss or humus layer approach the optimum for oviposition only one day in four during the summer months in central New Brunswick.

From the above consideration of the possible effect of temperature on the parasites' searching ability and oviposition, it follows that the parasitism of cocoons in the warm climate of southern New Brunswick should be higher than in the cooler northerly areas. There was evidence of this trend every year, but particularly in 1939, when *D. fuscipennis* attained its highest population. That year 53,000 sound cocoons of the sawfly were collected at 179 points in the Province. The parasitism by *D. fuscipennis* averaged 11.0 per cent of the total cocoons in southern New Brunswick and 2.9 per cent in northern New Brunswick.

Low temperatures at the onset of winter may cause some mortality, depending upon the development of the parasite at that time. Eggs laid after the last week of September, advanced (black) pupae, and unemerged adults generally do not survive the winter. About 1,000 parasitized sawfly cocoons were collected and dissected in late October and November, 1939, to determine the proportions of stages entering the winter. The percentages by stages were: Adults, 2.4 per cent; black pupae, 3.1 per cent; white pupae, 71.8 per cent; mature larvae and prepupae, 22.7 per cent; and eggs, 0 per cent. These and other records indicate that winter mortality does not generally exceed 5 per cent. Winter mortality is negligible among last instar larvae or pupae in diapause (white pupae). Samples of these stages stored outside above snow level until January 4, 1939, survived a minimum temperature of − 7.5° F. Daily ground temperatures (depth, about one inch) have been taken by C. C. Smith in a stand near Fredericton for over ten years. He found that the temperatures rarely drop below 30° F. except during late November or early December in the absence of snow.

TABLE 4.—INTRODUCED PARASITES REARED FROM COCOONS OF THE EUROPEAN SPRUCE SAWFLY COLLECTED FROM POPULATION PLOTS IN QUEBEC AND NEW BRUNSWICK. DISTANCES BETWEEN PLOTS AND PARASITE LIBERATION POINTS SHOWN IN TABLE 2.

Location of plots	Date collected	Sawfly cocoon population		Number cocoons reared	Percentage parasitism by species		
		Square foot forest floor	Sound cocoons per square foot		Total	*D. fuscipennis*	*Exenterus* sp.
Parke Reserve, Que.	1934, May	176	21.1	3,705	0	0	0
	1934, Oct.	32	18.5	591	0	0	0
	1935, May	168	13.3	817	0	0	0
	1935, Sept.	176	26.6	3,140	0	0	0
	1936, May	200	18.6	2,168	0	0	0
	1936, Oct.	176	34.6	5,835	0	0	0
	1937, Oct.	200	17.4	2,930	1.2	1.2	0.03
	1938, Oct.	176	13.2	1,388	1.7	0.1	1.6[1]
	1939, Oct.	200	12.2	1,802	8.7	5.9	2.8[2]
McNamee, N.B.	1934, Oct.	32	0.9	31	0	0	0
	1935, Oct.	196	0.8	150	0	0	0
	1936, Oct.	196	0.7	135	0	0	0
	1937, Oct.	196	2.6	511	0	0	0
	1938, Oct.	196	5.8	1,103	0	0	0
Dunbar, N.B.	1935, Oct.	188	1.7	320	0	0	0
	1936, Oct.	188	1.0	188	0	0	0
	1937, Oct.	188	9.3	1,748	0	0	0
	1938, Oct.	188	13.9	2,692	0.3	0.3	0
	1939, Oct.	188	6.7	1,333	5.2	5.2	0
	1940, Oct.	188	0.2	42	2.4	2.4	0
	1941, Oct.	188	0.015	3	33.3	33.3	0
	1942, Oct.	188	0.06	11	0	0	0
Canaan River, N.B.	1936, Oct.	200	12.3	2,410	0	0	0
	1937, Oct.	200	20.8	4,099	3.3	3.1	0.2[3]
	1938, Oct.	200	21.7	4,005	0.8	0.8	0.02[3]
	1939, Oct.	200	10.4	1,686	13.9	13.7	0.2[3]

[1] About 80 per cent *E. amictorius*, remainder *E. claripennis*.
[2] About 70 per cent *E. amictorius*, remainder *E. claripennis*.
[3] *E. vellicatus*.

TABLE 5.—INTRODUCED PARASITES REARED FROM COCOONS OF THE EUROPEAN SPRUCE SAWFLY COLLECTED FROM POPULATION PLOTS AT YOUNG'S BROOK, YORK CO., N.B., AND ACADIA FOREST EXPERIMENT STATION, SUNBURY CO., N.B. DISTANCES BETWEEN PLOTS AND PARASITE LIBERATION POINTS SHOWN IN TABLE 2. TOTAL OF 200 SQUARE FEET OF FOREST FLOOR EXAMINED EACH YEAR AT EACH LOCATION

Locality	Date collected	Sound cocoons per square foot	Number cocoons reared	Percentage parasitism by species			
				Total	*D. fuscipennis*	*Exenterus* sp.	*E. claripennis*
Young's Brook	1937, May	7.80	1,420	0	0	0	0
	1938, May	16.67	3,059	0.1	0.1	0	0
	1939, May	23.95	4,790	1.7	0.1	0.1[1]	1.5
	1940, May	7.16	1,433	4.9	2.0	0.1[1]	2.8
	1941, May	0.22	43	4.6	2.3	0	2.3
	1942, May	0	0	0	0	0	0
	1943, May	0.01	2	0	0	0	0
	1944, May	0.01	2	0	0	0	0
	1945, May	0.01	1	0	0	0	0
	1946, May	0.04	8	25.0	0	25.0[2]	0
	1947, May	0.02	4	25.0	0	0	25.0
	1948, May	0	0	0	0	0	0
	1949, May	0.01	2	0	0	0	0
	1949, Oct.	0.05	10	10.0	0	10.0[2]	0
	1950, Oct.	0.01	2	50.0	0	50.0[2]	0
	1951, Oct.	0.01	3	0	0	0	0
Acadia Station	1938, May	2.30	460	0	0	0	0
	1939, May	5.20	447	2.0	0	0	2.0
	1940, May	1.36	273	15.1	13.3	0	1.8
	1941, May	0.23	45	0	0	0	0
	1942, May	0.06	12	0	0	0	0
	1943, May	0.05	10	0	0	0	0
	1944, May	0.05	11	0	0	0	0
	1945, May	0.26	46	10.9[3]	0	4.3	0
	1946, May	0.02	4	25.0	0	25.0[4]	0
	1947, May	0	0	0	0	0	0
	1948, May	0.01	1	0	0	0	0
	1949, May	0.01	2	0	0	0	0
	1950, May	0.01	2	50.0	0	50.0	0
	1951, May	0.01	1	0	0	0	0
	1952, May	0.03	7	14.5	0	14.5[2]	0

N.B.—Because of the small numbers of cocoons reared in later years the percentages of parasitism are not necessarily significant.

[1] *E. amictorius.* [2] *E. vellicatus.* [3] Includes three specimens of *D. bohemica.* [4] One specimen of *E. tricolor.*

Finlayson and Reeks (16) showed that under laboratory conditions adults of the parasite sometimes fail to paralyse their host prior to oviposition, and as a result some eggs are ingested by the eonymph in the cocoon. Ullyett (33) attributes this failure to high evaporation, causing excessive loss of moisture from the body. This presumably deprives the poison glands of an adequate supply of poison. He did not observe this condition at high humidities. There is nothing to indicate that there have been important losses of eggs from low humidity under field conditions in the Maritime Provinces.

Density of Host Population.—The establishment of *D. fuscipennis* at various levels of host population is shown in Tables 4 and 5, which should be used in conjunction with Table 2 for information on dates and numbers liberated.

Initial establishment occurred at the Acadia Forest Experiment Station when the sawfly cocoon population was as low as 1.4 per square foot (Table 5). On the other plots the initial recoveries following parasite liberations occurred at much higher densities, the numbers of cocoons ranging from 13.9 to 20.8 per square foot.

The parasite was recovered from five of the six plots. The highest parasitism on the plots occurred in most cases in 1939 and followed the peak of the sawfly cocoon population by one to three years. The random cocoon collections also showed exceptionally high parasitism in 1939 (Table 6). The reasons for high parasitism that year were low sawfly emergence, resulting in an abundant supply of host cocoons throughout the summer; a warm summer with temperatures above average; and a high incidence of disease, causing a low population of new cocoons in the autumn. Thus, conditions for a population increase were more favourable to the parasite than to the sawfly.

Although the effectiveness of *D. fuscipennis* was most apparent when the sawfly population was declining, the success of the parasite is dependent on a high sawfly population. The parasite has not been recovered from the very light populations that have prevailed since 1941. However, an interesting record of survival is shown for the Dunbar plot (Table 4). Of three sound cocoons collected from 188 square feet of forest floor, one was parasitized. The parasite was not recovered from the other plots when the cocoon population fell below 0.2 per square foot, but at this level the degree of sampling was inadequate. The plot records indicate that a cocoon population of over one per square foot is generally necessary for the effectiveness, and possibly survival, of *D. fuscipennis*. The availability of cocoons of other species of sawflies may also be an important factor in survival. The parasite was recovered from three of 1,600 cocoons of the larch sawfly, *Pristiphora erichsonii* (Htg.) in 1939, and Baird (2), Gray (18), and Friend (17) have reported it attacking *Diprion frutetorum* (Htg.).

Spread

Intensive collections near a liberation point suggest that spread was relatively slow in the Province of Quebec (29). This is also suggested by the sampling on the Parke Reserve plot (Table 4), which shows that the parasite was four seasons spreading about half a mile to the plot. Spread was equally slow on the Dunbar plot in central New Brunswick, but in this

case establishment was hindered by a very low host population. The parasite was only two seasons spreading up to one mile on the Young's Brook plot. The records of greatest spread in time and distance were obtained from the plot at the Acadia Forest Experiment Station, where the liberations of *D. fuscipennis* totalled 1,000,000 specimens in 1938. In August, 1939, R. F. Morris collected all the sound cocoons beneath three spruce trees about 6 miles from the nearest liberation point, and found that 23 per cent of the cocoons were parasitized. The following spring 13.3 per cent of the cocoons from a plot in about the same area were parasitized, which is proof of the parasites' ability to spread 6.5 miles in two seasons. In central Gaspé *D. fuscipennis* was recovered only at, or a few yards from, the liberation points.

Spread was rapid under favourable conditions of climate and host density, and was slower in a cool climate or when the host density was low.

Distribution

Although *D. fuscipennis* was generally more abundant in central and southern New Brunswick, it was recovered in the Province from nearly all forest regions where the sawfly was numerous.

Positive proof of establishment in Nova Scotia is lacking, but a few cocoons showed the presence of emergence holes thought to have been those of *D. fuscipennis*. The parasite was not recovered from Prince Edward Island or Newfoundland. The poorer showing of the parasite in these provinces was undoubtedly due to light sawfly populations and inadequate sampling.

Aptesis basiconia (Grav.)

The habits of this cocoon parasite, and particularly the variety *curtulus* Kr., have been discussed by Morris *et al.* (25). They noted that females emerging from field collections at the normal time paralyse the host, prior to oviposition, and render it inactive, but in laboratory-bred individuals this power appears to get successively weaker. It is not known whether their sawfly material was *D. hercyniae* or *D. polytomum*, since the distinction between these species was not recognized at that time. The degree of paralysis associated with oviposition may be stronger in the latter species.

In Canada, the rearing of *A. basizonia* on *D. hercyniae* in field or laborarory cages has been contingent largely on the paralysis of the host by exposing the cocoons to heat, as shown by Finlayson and Reeks (16) and Green (19). This prevents the sawfly eonymphs from ingesting or crushing the parasite eggs. In field tests all attempts to rear laboratory-bred parasites on sound cocoons failed completely, but the rearing of a few specimens from field-collected cocoons proves that *A. basizonia* can develop successfully in normal sawfly cocoons. Successful development probably occurs only when the host is attacked in the pupal stage, which is less active than the eonymph or pronymph.

Adults of *A. basizonia* appear to be good searchers because they readily parasitized scalded sawfly cocoons covered with moss in field cages. Apparently the climatic conditions of Eastern Canada are favourable to the establishment of the parasite, as Baird (4) reported the recovery of nine specimens from Kamouraska County, Que., in 1939, which is more than the

total number recovered in the warmer regions of the Maritime Provinces during the entire period of study. However, the small numbers of specimens recovered is evidence of the relative ineffectiveness of this species as a parasite of *D. hercyniae*. A total of five specimens was recovered from cocoons collected at liberation points near Young's Brook and North Tay, York County, N.B., and the upper Cascapedia Watershed in the Gaspé Peninsula (Table 6). The first specimen was recovered in 1934 and the last in 1939. The parasite has not been found in Nova Scotia, Newfoundland or Prince Edward Island.

Exenterus claripennis (Thom.)

The establishment and behaviour of this larval parasite near two liberation points were discussed in an earlier paper by Reeks (31). The brief account that follows is confined to supplementary records. The seasonal development is shown diagrammatically under "*Exenterus* spp." in Figure 1.

Establishment

The early confusion in the taxonomy of the species of *Exenterus* (31) makes it difficult to relate the degree of establishment to the numbers of *E. claripennis* liberated. This species was recovered from three of the sawfly population plots. Its apparent failure to become established on

TABLE 6.—PARASITISM OF SOUND COCOONS OF THE EUROPEAN SPRUCE SAWFLY BY INTRODUCED SPECIES OF PARASITES. MAJORITY OF COCOONS COLLECTED AT RANDOM WITHIN ONE MILE OF PARASITE LIBERATION POINTS

Year	No. collections	No. cocoons	Percentage parasitism by species					
			Total		*D. fuscipennis*		*Exenterus* spp.	
			Average	Highest	Average	Highest	Average	Highest
New Brunswick								
1934	6	300	0.3[1]	1.9[1]	0	0	0	0
1935	6	420	0	0	0	0	0	0
1936	6	1,110	0	0	0	0	0	0
1937	26	24,350	1.5	5.1	1.3	5.1	0.2	1.4
1938	61	71,020	0.5[2]	64.5	0.2	64.5	0.3	4.9
1939	179	52,840	10.6[3]	73.6	10.3	73.6	0.3	7.9
1940	91	11,030	2.8	56.7	2.7	56.7	0.1	5.6
1941	45	870	3.3	30.3	2.0	30.3	1.3	16.1
Upper Cascapedia River, Gaspé Peninsula, Que.								
1934	23	8,870	0.04[1]	2.2[1]	0.01	0.3	0.03	0.8
1935	13	15,350	0.1	2.2	0.1	2.2	0.01	0.5
1936	3	1,900	0	0	0	0	0	0
1937	12	19,180	0.03	0.3	0.01	0.2	0.02	0.3
1938	10	5,270	0.3	1.6	0.2	1.0	0.1	1.6
1939	15	11,190	0.3	4.8	0.1	0.8	0.2	4.8
1940	4	1,770	5.9	17.4	0	0	5.9	17.4
1941	18	5,280	1.2	7.3	0	0	1.2	7.3

[1] One specimen *A. basizonia*.
[2] Two specimens *D. bohemica*.
[3] Two specimens *D. bohemica*; three of *A. basizonia*.
All *Exenterus* spp. for Cascapedia = *E. claripennis*.

TABLE 7.—SUMMARY OF INTRODUCED PARASITES REARED FROM LARVAE OF THE EUROPEAN SPRUCE SAWFLY COLLECTED AT RANDOM BY THE FOREST INSECT SURVEY IN NEW BRUNSWICK

Year	Number of collections	Sawfly larvae per tree sample, all instars	Sawfly cocoons reared from larval collections		Parasites reared			
			Sound*	Percentage parasitized	Total specimens identified	Percentages of total		
						D. bohemica	*E. claripennis*	*E. vellicatus*
1937	253	20.0	4·000	0	0	0	0	0
1938	525	13.0	3·000	0	0	0	0	0
1939	490	14.8	3·181	0	0	0	0	0
1940	432	6.7	449	0	0	0	0	0
1941	300	4.6	296	0	0	0	0	0
1942	321	1.4	144	0	0	0	0	0
1943	235	3.0	132	1.5	2	0	100.0	0
1944	190	2.1	520	1.5	2	0	50.0	50.0
1945	219	1.5	391	6.1	22	40.9	18.2	40.9
1946	253	1.6	586	5.5	23	74.0	13.0	13.0
1947	325	0.9	333	27.3	91	95.6	1.1	3.3
1948	268	0.7	309	32.4	101	96.0	0	4.0
1949	263	0.7	287	18.5	55	92.7	0	7.3
1950	277	0.6	246	29.3	72	93.0	0	7.0
1951	207	0.6	156	11.6	19	73.7	0	26.3

* Sound cocoons=Cocoons from which sawflies or parasites emerged; diseased specimens excluded; occasionally two *bohemica* will issue from one host.

two of the plots, at McNamee and Dunbar (Table 4), was probably due to the time of the year when the parasites were released (September 25), or to the small numbers released (Table 2). However, establishment has followed the liberation of very small numbers in some areas. In the Gaspé, the parasite became well established (Table 6) following the liberation of 140 specimens at one point and 110 at another. The establishment of *E. claripennis* and its related species is remarkable considering that they were released in much smaller numbers than those of the cocoon parasites.

The initial establishment of *E. claripennis* on the plots (Tables 4, 5) occurred when the sawfly cocoon population was as low as 5.2 per square foot. At the Acadia Forest Experiment Station, where parasites were liberated on the plot, small numbers were recovered the year of liberation, although the counts were made the following spring (Table 5). The highest parasitism occurred on the Young's Brook plot the fourth season following liberation, or one year following the peak of the sawfly cocoon population (Table 5). These and previous records (31) show that establishment is rapid at moderate to high levels of host population.

The parasite may not survive at extremely low levels of sawfly population. Only one specimen has been taken from all the plots after 1941 (Table 5); this was in 1947, which was the seventh year of very low sawfly population.

A similar trend of low survival under conditions of light host density is indicated by the sawfly larval sampling. The larval samples of the Forest Insect Survey, which are taken at random, show a fairly steady decline in sawfly numbers from 1937 to 1951, with the greatest drop in 1940. The first specimens of *claripennis* were recovered from these collections in 1943, and small numbers were taken each year up to and including 1947 (Table 7). These recoveries were associated with average sawfly larval populations of 0.9 to 3.0 per tree sample. The results of sampling by the same method were similar at the Acadia Forest Experiment Station. Here the parasite was taken in 1945 when the sawfly larval population ranged from 2.7 to 7.6 per tree sample (Table 8). In both series *E. claripennis* was not recovered after the larval population fell below 0.9 per tree sample. At larval population levels below this point the expectancy of survival for *E. claripennis* is lower than for *E. vellicatus* or *D. bohemica* (Tables 7, 8).

Distribution

E. claripennis is the only introduced species of *Exenterus* that has been taken in central Gaspé, but this undoubtedly is due to the distribution of colonies liberated rather than to climatic influence. It has been widely distributed in New Brunswick (Figure 2), particularly in the central region. It has not been recovered from the other Maritime Provinces, possibly because of the consistently lower sawfly populations.

Exenterus amicatorius (Panz.)

Of the *Exenterus* group, *E. amictorius* was second to *E. claripennis* in numbers recovered. The majority were reared from random collections. Only on the Parke Reserve plot (Table 4) did it appear particularly promis-

TABLE 8.—PARASITISM OF THE EUROPEAN SPRUCE SAWFLY AT TWO LOCATIONS ON THE ACADIA EXPERIMENT STATION, SUNBURY CO., N.B. LOCATION 1 ABOUT 300 YARDS AND LOCATION 2 ABOUT FOUR MILES FROM NEAREST PARASITE LIBERATION POINT, BUT *D. bohemica* RELEASED OVER 16 MILES FROM THE TWO SAMPLING LOCATIONS. (SEE TABLE 2 FOR PARASITE LIBERATIONS).*

Year	Location	Sawfly larvae per tree sample	Parasitism of sawfly reared from instars V and VI		Parasite species reared from all instars				
			Hosts reared	Percentage parasitized	Total specimens	Percentages of total			
						D. bohemica	*Exenterus* sp.	*E. claripennis*	*E. vellicatus*
1945	1	2.7	83	60.3	52	78.9	1.9	5.8	13.4
	2	7.6	73	43.8	39	66.7	18.0	2.5	12.8
1946	1	0.4	34	52.9	18	94.5	0	0	5.5
	2	0.8	66	54.5	36	83.4	5.5	0	11.1
1947	1	0.2	28	43.0	12	100.0	0	0	0
	2	0.3	14	39.0	20	75.0	0	0	25.0
1949	1	0.9	70	56.3	40	90.0	7.5	0	2.5
	2	0.9	115	77.0	67	88.2	4.4	0	7.4
1950	1	0.2	17	17.6	3	33.4	0	0	66.6
	2	0.5	58	36.0	27	92.2	3.9	0	3.9
1951	1	0.7	49	14.3	7	57.1	0	0	42.9
	2	1.4	58	24.1	14	78.6	0	0	21.4

* Data provided by F. T. Bird and D. E. Elgee.
N.B.—Sawfly larvae that died from disease are excluded from totals in computing parasitism. Sawfly collections made about twice weekly from June to September.

ing as a larval parasite. Rearings from field collections indicate that the seasonal development is much the same as that of *claripennis* (Figure) 1.

E. amictorius seems to be particularly dependent on host density. It was last taken on the Young's Brook plot when the cocoon population was about 7 per square foot, and was never recovered at lower levels of sawfly population. It was not reared from the larval collections at high or low sawfly densities. The evidence (Tables 7, 8) points to *amictorius* being more density-dependent than *E. claripennis* or *E. vellicatus*.

This parasite showed about the same rate of spread as *E. claripennis*. On the Parke Reserve plot (Table 4) it was four seasons spreading up to half a mile from the liberation point to the sampling areas. On the Young's Brook plot it took two years to spread about the same distance.

The distribution of *E. amictorius* in the Maritime Provinces is confined to central New Brunswick (Figure 2).

Exenterus vellicatus (Cush.)

E. vellicatus was described as new in 1940 by Cushman (13), who considered that it was the same species discussed under *Exenterus* sp. by Morris *et al.* (25) in 1937. Recoveries of this species were rare during the sawfly outbreak, but since 1940 the numbers recovered have exceeded those of the other species of *Exenterus*.

The life history has never been described. The following brief notes on the rearing of parasites from sawfly collections will show that its development is much the same as that of *E. claripennis* (Figure 1). The parasite has one and a partial second generation. First generation adults emerged from June 20 to July 5, the mean date for males being June 20 and for females July 1. Second generation adults emerged from the end of July to the third week of August. About 50 per cent of the summer generation remained in dispause in the host cocoon until the following spring. Parasitized larvae have been collected from July 3 to October 1. The eggs are laid on the integument of the host larva. The fifth and sixth instars are the most commonly attacked, but occasionally fourth instar larvae are parasitized. Hatching normally occurs within the host cocoon.

Establishment

E. vellicatus was recovered from two of the plots. It became established on the Canaan River plot (Table 5) during the peak of the sawfly population. The most significant point of this record is that establishment was effected from the release of a single colony of 65 male and female adults. Establishment was slower on the Young's Brook plot, presumably due to the small numbers released. The numbers reared from this plot were small, but the parasite was recovered three years when the sawfly cocoon population was less than 0.1 per square foot (Table 5). This indicates that it is more capable than *E. claripennis* or *E. amictorius* of maintaining itself at very low sawfly densities. This is substantiated by the two series of larval samples (Tables 7, 8), which show that *E. vellicatus* persisted at low sawfly densities and was recovered when the average host larval population was as low as 0.2 per tree sample.

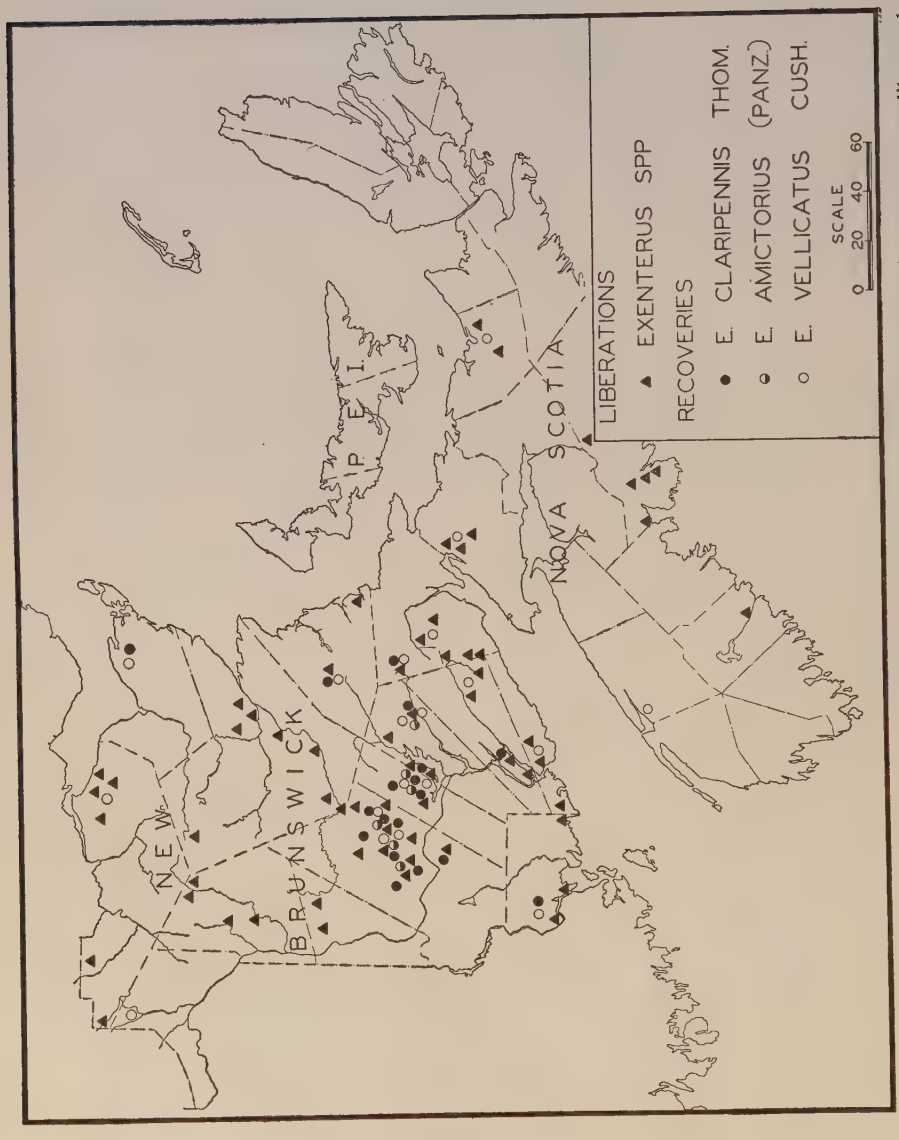

FIGURE 2. Approximate locations in New Brunswick and Nova Scotia where species of *Exenterus* were liberated and recovered. Each recovery point indicates one to many collections from which *Exenterus* spp. were reared. (Drawn by L. E. Williams).

Spread

The parasite spread from a liberation point to the sampling areas on one plot, distances of one-quarter to one-third of a mile, in three seasons. From seven to eleven years after liberations at the Acadia Forest Experiment Station, it showed about the same degree of abundance four miles from the liberation point as at the immediate point of liberation (Table 8). Striking records of spread were obtained in Newfoundland and Nova Scotia. In 1949, an adult of *E. vellicatus* was reared from a collection consisting of a single fifth-instar sawfly larva collected near Rattling Brook, Newfoundland. The sawfly was extremely rare in this area. The nearest *Exenterus* liberation point was near Millertown, 50 miles distant, where 395 individuals were released as *E. amictorius* in 1943.

A specimen was recovered in 1951 from a small collection (8 sawfly cocoons reared from field-collected larvae) taken at Middle Arm, White Bay District, Newfoundland. The nearest parasite liberation point was at Chouse Brook, where 87 parasites were released as *E. amictorius* in 1946. This record indicates that *E. vellicatus* spread at least 30 air-miles in five years. In 1951 a specimen was recovered near Seaview, Annapolis County, N.S. This suggests a spread of at least 55 miles from the nearest liberation point near Milton, Queens County, where 126 male and female parasites were released as *E. amictorius* in 1942. The distances shown for these three records of spread are conservative. There is no proof that the recovered specimens originated from the nearest parasite liberation point, which in each case was associated with *amictorius*. Since the latter species was clearly recognized as a distinct species by 1942, it is likely that colonies bearing this name were correctly labelled, and the recovered specimens of *E. vellicatus* may have originated from more distant liberation points than those indicated.

Distribution

This parasite has been recovered at a large number of widely separated points in New Brunswick (Figure 2). The specimens recovered in Nova Scotia and Newfoundland were the only representatives of the introduced *Exenterus* spp. that have been taken in these provinces. This is additional evidence that *vellicatus* is one of the most efficient species in surviving under conditions of very low host density.

Exenterus tricolor (Rmn.)

Only one specimen was recovered. It was reared from a cocoon collected in May, 1946, from the plot at the Acadia Forest Experiment Station (Table 5), where 188 individuals of *tricolor* were released in 1938.

Drino (*Prosturmia*) *bohemica* (Mesn.)

Mesnil's (21) work on the Sturmiinae suggests that up to five species of *Drino* may have been released against the sawfly in Canada. Only one species, *D. bohemica*, has been recovered.

Seasonal Development

The following summary of observations on development is based on records of rearing field-collected larvae.

The parasite has one and a partial second generation in central New Brunswick. Adult emergence commences about the latter part of June. First generation eggs or larvae are present from about the first week to the fourth week of July, and second generation eggs or larvae from the second week of August to the end of September (Figure 1). Under insectary conditions the emergence of 102 adults from larval collections occurred from July 26 to October 10, August 9 being the mean date. About half of 228 adults emerged during the summer of collection, the remainder the following year. The proportion of males and females was approximately equal. Over 90 per cent of the sawfly larvae bearing parasite eggs were in the fifth instar. The parasite has also been recovered from larvae collected in the fourth and sixth instars, and one specimen was reared from a larva that was collected in the first instar.

Upon the completion of feeding, the maggot issues from the empty host skin. This may occur when the dead host is in the fifth or sixth instar, but in 76 per cent of 289 observations the maggot issued from the sawfly remains in the host cocoon. Pupation usually occurs outside, but sometimes inside the sawfly cocoon. The method of pupation shows the inadequacy of cocoon sampling for the presence of this species. Some of the parasites overwinter as larvae within the host, but the majority as puparia in the forest floor.

Establishment

Briand (12) noted that the initial establishment of this species was slow near the Parke Reserve plot, where the parasite was first released in 1934. He first recovered the parasite in 1937, but it was not until 1942 that he noted a striking increase in its population and spread. Annual increases in parasitism continued until 1945, which was the last year in which observations were recorded (12).

The few specimens recovered during the sawfly outbreak in New Brunswick indicate that establishment of *D. bohemica* was much slower than that of most of the other introduced species. Sawfly larvae were collected once or twice a week from 1937 to 1941 at Young's Brook and from 1937 to 1951 at the Acadia Forest Experiment Station. These did not produce any *D. bohemica* until 1945, when the parasite became well established in the latter area (Table 8). This was also the first year of recovery from the Forest Insect Survey collections (Table 7).

This parasite has been recovered from larval collections every year since 1945, and has surpassed all other larval parasites in its ability to survive at low densities of sawfly population. Fairly high percentages of parasitism have occurred frequently even when the sawfly population was less than one per tree sample.

There are few significant records showing the effect of multiparasitism on the establishment of *D. bohemica*. However, multiparasitism of the sawfly has occurred occasionally, *D. bohemica* generally being successful over *Exenterus* sp. (31). In one unusual case an adult of *D. bohemica* and one of *E. vellicatus* were successfully reared from a sawfly larva.

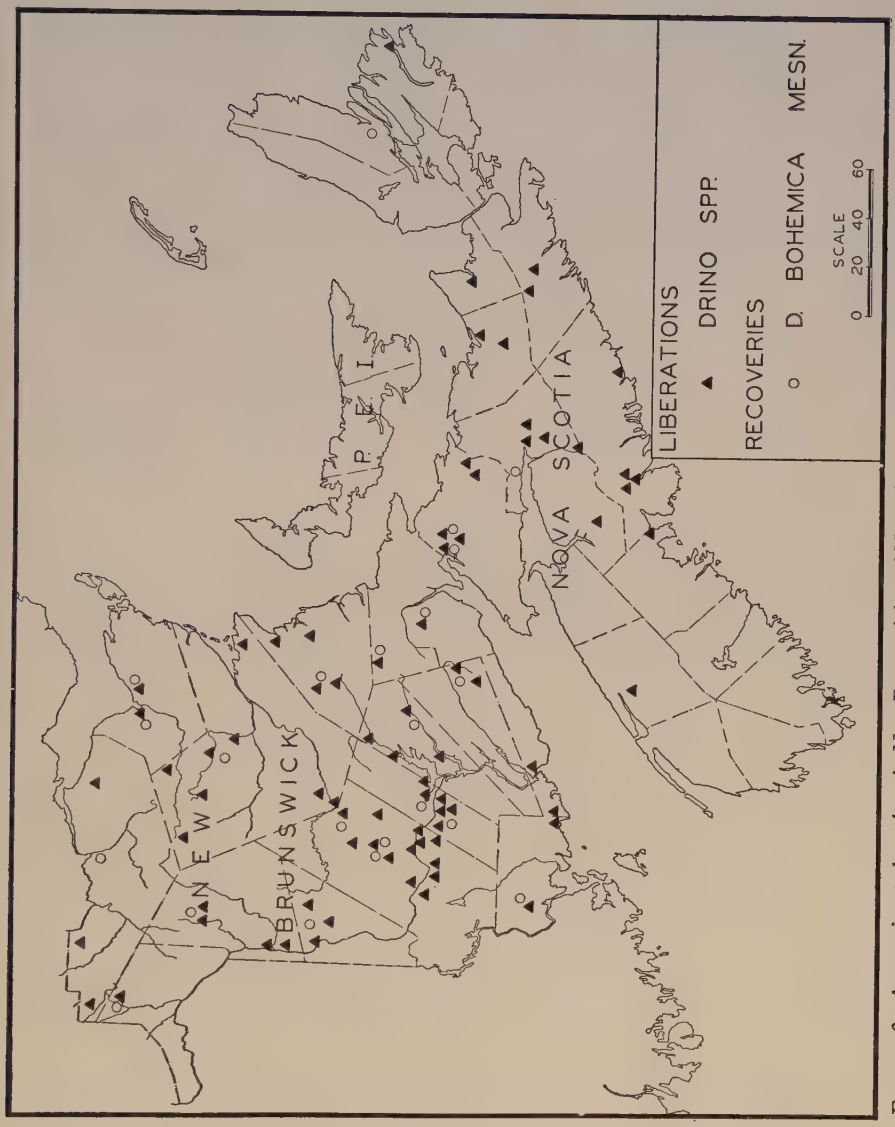

FIGURE 3. Approximate locations in New Brunswick and Nova Scotia where species of *Drino* were liberated and *D. bohemica* recovered. Each recovery point indicates one to many collections from which *bohemica* was reared. (Drawn by L. E. Williams).

D. bohemica has been reared in small numbers from *Pikonema alaskensis* (Rohwer) and *P. dimmockii* (Cres.). Its ability to attack other hosts may be an important factor of survival during a period of very low numbers of *D. hercyniae.*

Spread

Table 8 shows that trends in parasitism were similar at two sampling stations. These were about four miles apart, and located at the Acadia Forest Experiment Station, Sunbury County, N.B. The nearest *Drino* liberation point was about 8 miles from the two stations. However, liberations at this point were made in 1945, which was the first year of high parasitism, so it is assumed that the specimens reared originated from more distant liberations. The liberations in the region were made over a period of several years starting in 1934 at points ranging from 16 to 25 miles from the two sampling stations. Hence, it appears that *D. bohemica* reached a high level of population and dispersion about 11 years after the initial release in the region. Also, it is able to maintain its efficiency at low population levels.

Distribution

The parasite is widely distributed in New Brunswick, but has been taken at only four points in Nova Scotia (Figure 3) and one in western Newfoundland.

DISCUSSION

The sampling was not sufficiently adequate to permit very accurate estimates of the control value of the introduced parasites. The results are given simply in percentages of parasitism, as defined above. The percentages underestimate the control value of the parasites, but they indicate the relative importance of the various species.

Since surviving spruce trees showed first signs of recovery as early as 1940, it is of interest to review the parasite situation during and following the outbreak. Balch and Bird (10) showed that in 1939, and as late as 1941, the larval disease was the principal control factor, and was capable of reducing the sawfly numbers regardless of other factors. They noted that parasites may have been capable of bringing the "average" generation mortality above the 97 per cent necessary to prevent increase before scarcity of foliage and dying of trees added the factor of starvation. This necessary mortality, however, was accomplished largely by the sawfly disease, and other factors were considered to be of minor importance during the period of the epidemic of the virus. One of the parasites that contributed to reducing the severity of the outbreak, however slightly, was *D. fuscipennis*, followed or exceeded by *E. claripennis* in some areas. Their order of importance perhaps would have been reversed had they been liberated in equal numbers.

The importance of these two parasites decreased soon after the end of the outbreak. It is likely that the value of other biotic factors also has been changing gradually since detailed investigations of the sawfly terminated. The examination of sawfly larvae received by the Forest Insect Survey since 1947 indicates that the incidence of disease has been

lower than that reported earlier (10). Also, it is unlikely that the control value of small mammals is the same during the period of endemic sawfly numbers as it was during the outbreak. Since the end of the outbreak, *D. bohemica* has been one of the most important control factors of the sawfly, and *E. vellicatus* has been of some value.

The persistence and spread of these two species from 1941 to 1951, despite extremely low host populations, suggests that they are valuable permanent additions to the control complex and may be capable of preventing another sawfly outbreak, in combination with other control factors. They may be of particular importance when low populations of the host reduce the effectiveness of the disease.

The parasites *E. amictorius*, *E. tricolor*, and *A. basizonia* never showed great promise of being effective parasites either during or following the outbreak. However, it is recognized that the two former species were not released in large numbers.

It is clear that in the establishment of the introduced parasites, there have been various stages of parasite succession, with *D. bohemica* perhaps being the most effective during the endemic period of the sawfly. This succession seems to have been largely controlled by host density, but was influenced to some extent by the numbers of each species liberated.

SUMMARY

Seven species of introduced parasites have been recovered from the European spruce sawfly, *Diprion hercyniae* (Htg.), in the Maritime Provinces. These are the cocoon parasites *Dahlbominus fuscipennis* (Zett.) and *Aptesis basizonia* (Grav.); and the larval parasites *Exenterus claripennis* Thom., *E. amictorius* (Panz.), *E. vellicatus* Cush., *E. tricolor* Rmn., and *Drino* (*Prosturmia*) *bohemica* Mesn. The establishment, spread, and distribution of each species are discussed.

The most effective species have been *D. fuscipennis* and *E. claripennis* under conditions of high host density and *D. bohemica* and *E. vellicatus* at lower densities. However, the two latter species may be equally effective at higher levels of density providing they have a sufficiently long period for multiplication prior to a peak of sawfly population.

The species that showed the most rapid establishment were *D. fuscipennis*, *E. claripennis*, and *E. amictorius*. The two first species were particularly capable of attacking fairly large numbers of sawflies the year of liberation, providing host numbers were adequate. *D. bohemica* and *E. vellicatus* required a longer period before they became well established. *E. vellicatus* spread at least 50 miles in seven seasons, *D. fuscipennis*, $6\frac{1}{2}$ miles in two seasons, and *D. bohemica*, 16 to 25 miles in 12 seasons.

ACKNOWLEDGMENTS

Large numbers of reared parasites were identified by specialists of the Systematic Unit, Division of Entomology, Ottawa. G. S. Walley determined several hundred specimens of *Exenterus* spp. W. R. M. Mason and A. R. Brooks identified representative series of *D. bohemica*. L. R.

Finlayson, of the Dominion Parasite Laboratory, examined several series of *E. claripennis* and *E. amictorius*. This assistance is gratefully acknowledged.

The project was initiated by R. E. Balch, Officer-in-Charge of the Laboratory of Forest Zoology, Fredericton, N.B., whose criticisms have been most helpful. The frequent advice of A. B. Baird, Head, Biological Control Investigations, Ottawa, is appreciated. Many of the records of parasitism at the Acadia Forest Experiment Station were provided by D. E. Elgee. Acknowledgment is also made of the information provided by others who have been associated with the sawfly investigations, particularly R. F. Morris, M. L. Prebble, C. C. Smith, L. J. Simpson and F. G. Cuming.

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THE USE OF A DIFFUSION CHAMBER AS A MEASURE OF THE RATE OF OXYGEN SUPPLIED BY A SOIL[1]

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ABSTRACT

A study was made on the use of the Raney diffusion chamber in determining the effect of soil moisture and temperature on the rate of oxygen diffusion and in measuring differences in the oxygen-supplying power of field soils under different treatments. The results indicate that the moisture content of the sample and temperature affect the rate of oxygen diffusion. Measurements of diffusion rate should be made at moisture contents below the moisture equivalent value and at temperatures between 60° F. and 90° F. The data show a much higher diffusion rate in a Fox sand than in a Brookston clay. Tillage treatments on a Lockport soil (29.5 per cent sand, 42.2 per cent silt, 24.3 per cent clay) influenced the oxygen diffusion rate which was highest where the mouldboard plough, one-way disk plus mouldboard plough and the disk plough treatments were used. The use of rotary tillage equipment significantly reduced the oxygen-supplying power of this soil.

INTRODUCTION

The movement of gases between the soil and the atmosphere occurs by mass flow or streaming of air and diffusion. Mass flow results from gradients in the total pressure between the soil air and the atmosphere. Pressure gradients of this type may be due to temperature differences between the soil and the atmosphere, to barometric changes, to the effect of wind blowing over the soil surface or to changes in the volume of pore space available for air as a result of rain and evaporation of soil moisture. Keene and Rommell (2, 5) concluded that the interchange of gases between the soil and the atmosphere as a result of mass flow is of minor importance. Only the process of diffusion, therefore, will be considered important in bringing about an interchange of gases between the soil and the atmosphere.

Individual gases diffuse in response to their own partial pressure gradients. Diffusion of carbon dioxide out of, and oxygen into the soil tends to produce an equality of composition between the soil air and the atmosphere. In field soils, particularly during the growing season, considerable quantities of oxygen are consumed and appreciable amounts of carbon dioxide are produced by biological processes. As a result, the partial pressure of oxygen in the soil is reduced below that of the atmosphere and the partial pressure of carbon dioxide is increased above its normal atmospheric pressure. Because these partial pressure gradients tend to be maintained or accentuated by biological processes, there is a continuous interchange of oxygen and carbon dioxide between the soil and the atmosphere by diffusion.

Buckingham, Penman and Taylor (1, 3, 7) established the rate of diffusion as a function of the volume of air-filled pores. The general diffusion law may be expressed as:

$D = Do\ f(S)$ where D = diffusion coefficient through a porous material or air-filled pore space (S)

Do = coefficient of diffusion in free air when $S = 1.0$

[1] Contribution from the Department of Soils, Ontario Agricultural College, Guelph.
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Buckingham (1) concluded that $f(S) = S^2$. Subsequently Penman (3) established that the relationship of rate of diffusion to air-filled porosity is linear over a wide range of diffusion rates and porosities. Russell (6) states that the validity of this statement is limited to those instances where the volume of air-filled voids is a valid measure of the volume of such voids accessible for diffusion. In some instances it may be in error because of the existence of isolated soil pores cut off from direct connection to the atmosphere by water-filled necks. Taylor (7) showed that oxygen diffusion was influenced by degree of compaction and moisture content of soil samples inasmuch as these variables influence the total air-filled pore space of the sample. Because the rate of diffusion is proportional to the square of the absolute temperature (2), extremes of temperature must also be considered.

A diffusion chamber for measuring the rate of oxygen diffusion in the field has been designed and described by Raney (4). The object of this investigation was to study the use of the Raney diffusion chamber in determining the effect of soil moisture and temperature on the rate of oxygen diffusion. This report also discusses the applicability of the chamber for measuring differences in the oxygen-supplying power of field soils under different treatments. If this be a valid method, then a field method of measuring the degree of soil aeration, which in turn is related to other physical properties, becomes available.

EXPERIMENTAL PROCEDURE

In order to determine the effect of soil moisture on the rate of oxygen diffusion, diffusion chambers were placed on a three-foot grid pattern in a uniformly cultivated Burford loam soil to a depth of five inches. The area under study was thoroughly wetted to increase the moisture content well above field capacity. To prevent rapid losses of water by evaporation and further wetting by rain, the area was covered by a canvas. At intervals of two to three days, diffusion and moisture measurements were made as the plot gradually dried out.

To investigate the effect of temperature on the rate of diffusion, the diffusion chambers were filled with nitrogen gas and gaseous diffusion was allowed to take place in air at various temperatures.

The diffusion chambers were used under field conditions to measure the rate of oxygen diffusion in a Fox sand and a Brookston clay which had received different soil treatments. The study was designed to compare diffusion rates in soils of different textures and to evaluate the applicability of the method in characterizing soil aeration conditions within a textural class.

The treatments selected on the Fox sand were those that might cause an increase in the concentration of carbon dioxide in the soil air by excluding atmospheric oxygen or by the processes associated with the decomposition of organic matter. The treatments studied were:

(*a*) No cultivation which resulted in surface crusting and cracking;
(*b*) Thirty tons of manure per acre incorporated into the soil plus a surface mulch of straw 4-6 inches in depth;
(*c*) Commercially available rockwool insulation, four inches thick on the surface, and
(*d*) Thirty tons of manure per acre incorporated into the soil.

The plots were planted to Katahdin potatoes.

The area of Brookston clay under study exhibited many characteristics of heavy soils that have been intensively cropped with corn and soybeans. Improvement in soil aggregation and porosity has been noted following the use of rotations that include at least two years of a grass-legume mixture. The plots of corn examined by the diffusion probes were part of the following three-year rotations:

(*a*) Corn, oats and sweet clover. Manure was applied prior to corn planting;
(*b*) Corn, oats and alfalfa;
(*c*) Corn, oats and sweet clover;
(*d*) Corn, soybeans and soybeans;
(*e*) Corn, oats seed to sweet clover and soybeans. The sweet clover was ploughed under in the fall of the same year in which it was seeded.

The diffusion chambers were used in a Lockport soil in an attempt to measure differences in soil aeration brought about by different tillage treatments. This Lockport soil, on which a tillage experiment was established, contained 29.5 per cent sand, 46.2 per cent silt, and 24.3 per cent clay by the Bouyoucos method and had a moisture equivalent value of 22.5 per cent. The rates of oxygen diffusion were measured on the fall wheat plots in a rotation of hay-hay-fall wheat-red clover-corn-oats.

In preparing the land for fall wheat, the following six tillage treatments were used:

(*a*) Disk plough;
(*b*) One-way disk followed by mouldboard plough;
(*c*) Mouldboard plough;
(*d*) Rotary tillage A;
(*e*) Rotary tillage B;
(*f*) One-way disk.

Following the above basic tillage treatments all the plots were double-disked and harrowed lightly. Measurements were taken in the fall four weeks after planting and at the end of April. As an aid in interpreting the rates of diffusion, bulk density and moisture contents were determined.

DISCUSSION OF RESULTS

Effect of Moisture on Diffusion Rates

The study of soil moisture vs. diffusion rate, was conducted on a Burford loam having the following properties:

(*a*) Mechanical composition (Bouyoucos method)

Sand	40.5 per cent
Silt	44.9 per cent
Clay	14.6 per cent
(*b*) Moisture equivalent	24.0 per cent
(*c*) Wilting point	13.0 per cent
(*d*) Organic matter	6.0 per cent

The data in Figure 1 show the relationship of diffusion rate to moisture content within the moisture range of the experiment. At a moisture content slightly higher than the moisture equivalent these data indicate that the diffusion rate begins to increase rapidly and as the soil dries to the moisture equivalent value the rate of diffusion continues to increase. At a moisture content slightly lower than moisture equivalent the slope of the diffusion rate curve begins to decrease and finally approaches zero. It is apparent that this curve cannot be exactly duplicated on another soil, but it is believed that a similar curve having a flex point close to the moisture equivalent value can be plotted for most soils. It is suggested that the

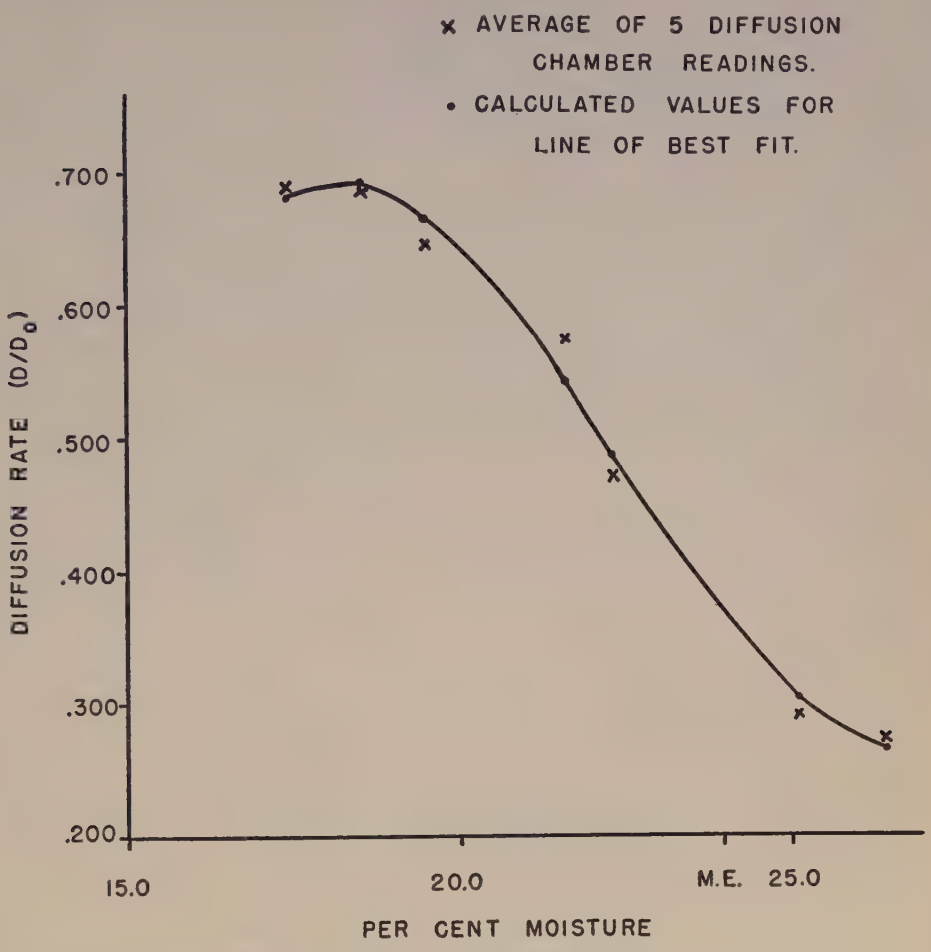

FIGURE 1. Effect of moisture on rate of diffusion in a Burford loam soil.

nature of this curve is closely related to pore-size distribution and, therefore, the nature of the moisture desorption curve.

The curve drawn in Figure 1 was calculated to be the line best fitting the experimental values. It is incorrect to assume that by using the equation of this line diffusion rate values at moisture contents above or below the experimental range can be determined.

From the data in Figure 1 it is concluded that at moisture contents between 17 and 20 per cent the rate of oxygen diffusion in this soil will not be significantly affected by soil moisture content. Experience has indicated that these limits of moisture content may be expanded to include the moisture range from slightly above permanent wilting point to slightly below the moisture equivalent value.

Effect of Temperature on Diffusion Rate

Because the oxygen analyser is calibrated at 75° F., the rate of oxygen diffusion at this temperature in open air from a diffusion probe was used as a starting-point in plotting a theoretical curve of diffusion as a function of

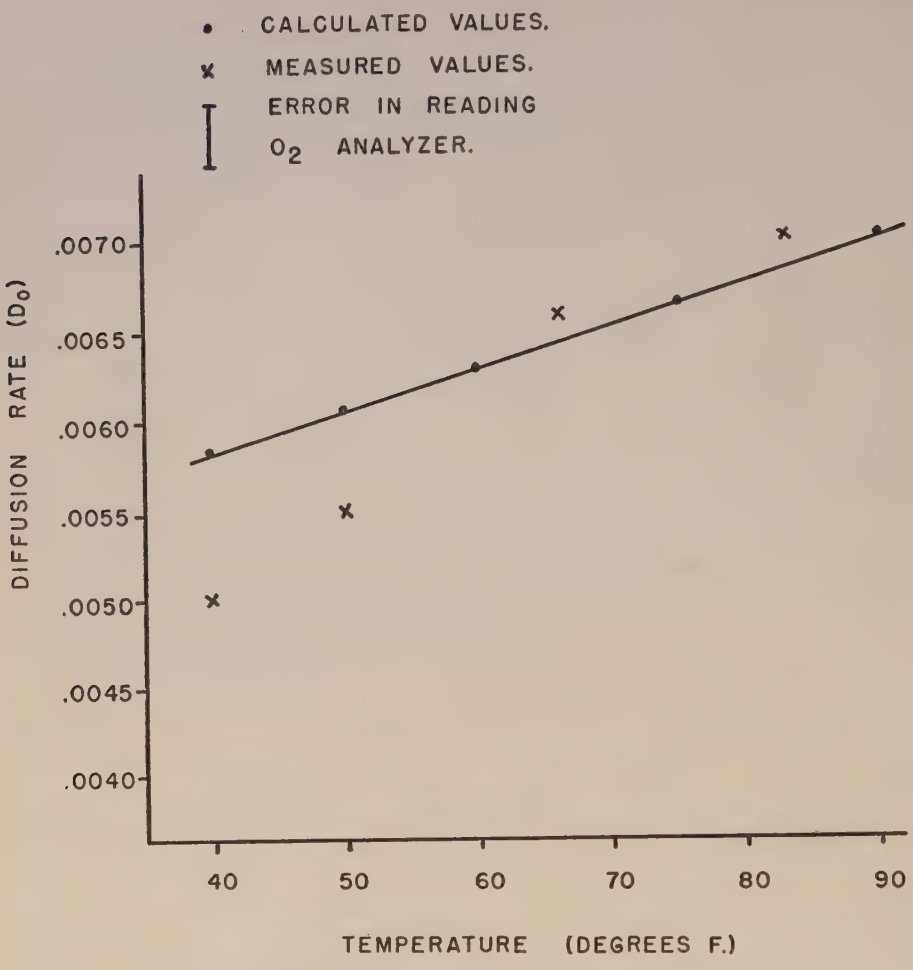

FIGURE 2. Effect of temperature on rate of oxygen diffusion.

temperature. The rate of oxygen diffusion measured at different temperatures agrees fairly well with theoretical rates. As there is an error in reading the analyser of $\pm$ 0.25 per cent oxygen, the measured diffusion rates reported in Figure 2 fall within or close to the error limits. These data indicate that, between 60° F. and 90° F., the effect of temperature on diffusion rates is within experimental error.

Diffusion Rates in Soils Under Different Treatments

Table 1 shows the diffusion rates in a Fox sand and in a Brookston clay under different treatments.

Diffusion measurements in the sand and the clay soils illustrate the differences in the rate at which these soils supply oxygen to an area where diffusion is occurring.

The diffusion rates recorded in Table 1 were obtained by using three diffusion chambers per replicated plot and averaging the individual determinations. A statistical interpretation of the effect of different treatments

TABLE 1.—THE RATES OF OXYGEN DIFFUSION IN A FOX SAND AND A BROOKSTON CLAY

Fox sand		Brookston clay	
Treatment	Diffusion rate	Treatment	Diffusion rate
No. Cult.	0.970	C-O-S.S. + Man.	0.696
Man. + Straw	0.878	C-O-Alf.	0.651
Rockwool	0.871	C-O-S.C.	0.638
Manure	0.833	C-Soy-Soy.	0.597
		C-O + S.C.-Soy.	0.583
Mean	0.888	Mean	0.633

on diffusion rates was not attempted as the study was intended to indicate differences between soils and within treatments on a given soil.

Use of the Diffusion Chambers to Measure the Effect of Different Tillage Practices

The data collected at both sampling dates were statistically analysed but only the diffusion rates showed significant differences between tillage treatments (Tables 2 and 3). The differences in diffusion rates measured in the fall and those measured in the spring might be attributed, in part, to climatic effects on the types of soil structures left by the tillage implements.

It is evident that the best treatments as measured by rate of oxygen diffusion in the soil are the disk plough, mouldboard plough and the one-way disk plus mouldboard plough. The first four treatments listed in Table 2 are not significantly different nor are the last four.

In Table 3 the diffusion rate measurements made in the spring are presented. The mouldboard plough treatment is significantly better than all other tillage treatments and the mouldboard plough, the disk plough, and the one-way disk plus mouldboard plough treatments promoted the highest rates of diffusion. The one-way disk plus mouldboard plough, disk plough, and one-way disk treatments were not significantly different, but all promoted significantly greater diffusion rates than the rotary tillage treatments.

TABLE 2.—OXYGEN DIFFUSION ON TILLAGE PLOTS 4-INCH SAMPLING DEPTH IN WINTER WHEAT, OCTOBER 1951

Treatment	Per cent moisture	Bulk density	Diffusion rate
Disk Plough	20.7	1.24	0.576
One-way Disk + Mould-Plough	21.1	1.25	0.541
Mouldboard Plough	21.9	1.26	0.481
Rotary Tillage A	21.4	1.22	0.438
Rotary Tillage B	20.5	1.21	0.372
One-Way Disk	21.3	1.23	0.291
Mean	21.1	1.23	0.460
L.S.D. 5 per cent	N.S.	N.S.	0.192
1 per cent	N.S.	N.S.	0.273

TABLE 3.—OXYGEN DIFFUSION ON TILLAGE PLOTS 4-INCH SAMPLING DEPTH IN WINTER WHEAT, APRIL 1952

Treatment	Per cent moisture	Bulk density	Diffusion rate
Mouldboard Plough	19.6	1.29	0.521
One-Way Disk + Mouldboard Plough	20.5	1.27	0.476
Disk Plough	19.5	1.29	0.473
One-Way Disk	18.6	1.31	0.471
Rotary Tillage A	20.4	1.28	0.416
Rotary Tillage B	20.0	1.26	0.402
Mean	19.9	1.28	0.450
L.S.D. 5 per cent	N.S.	N.S.	0.037
1 per cent	N.S.	N.S.	0.052

These data agree with the data of Raney (4) that showed the diffusion rate to decrease in order with the following treatments—regular plough > disk > rotary tiller.

CONCLUSIONS

The data presented indicate that the moisture content of the sample and temperature affect the rate of oxygen diffusion as measured by this diffusion chamber. Measurements of oxygen diffusion rates should be made only at moisture contents below the moisture equivalent, and at air temperatures between 60° F. and 90° F., assuming the soil has come to equilibrium with the air temperature.

This diffusion apparatus was used to measure differences in the rate of oxygen diffusion due to soil texture and cropping practices. The rate of diffusion in a Fox sand is shown to be greater than in a Brookston clay soil. The use of the diffusion chamber to measure differences in diffusion rate due to different tillage treatments on the Lockport soil showed the diffusion rate to be highest where the mouldboard plough, one-way disk plus mouldboard plough, and the disk plough treatments were used. The use of rotary tillage equipment significantly reduced the oxygen-supplying power of the Lockport soil.

Experience has indicated that a number of readings must be taken within a plot to overcome variability in aeration conditions and to satisfy statistical requirements.

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STUDIES IN RED CLOVER SEED PRODUCTION. PART II[1]

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ABSTRACT

Studies on red clover seed production as related to insect control, pollination and nectar secretion are reported. These studies were supplementary to those carried on in 1950.

The insecticides, DDT and Toxaphene, controlled the pea aphid and tarnished plant bug but the control of these insects did not result in increased seed yields. The insecticides failed to control the red clover seed midge.

Pollinator populations of both honeybees and bumblebees varied significantly from day to day and were significantly higher in the afternoon than in the morning. Honeybees and bumblebees were present in the ratio of 2.2 : 1 when two colonies of honeybees per acre were used. Bumblebees were dispersed uniformly over the experimental area.

The importance of colony placement was indicated by the fact that (*a*) the number of honeybee pollinators decreased as the distance from the apiary increased, (*b*) the greater the number of honeybee pollinators, the greater the seed yield, and (*c*) seed yield decreased as the distance from the apiary increased.

There was a great variation in the amounts and kinds of pollen gathered by individual colonies. Red clover pollen collections ranged from 11 to 71 per cent. On the basis of all pollen gathered that of red clover was third in order of importance being 24 per cent of the total weight. Day-to-day comparisons showed that the amount of this pollen collected increased from 4 to 83 per cent from the first to the last day of the test.

INTRODUCTION

Recent studies with red clover by MacVicar *et al.* (4) on the control of harmful insects, pollination and nectar secretion indicated that: (*a*) seed yields were appreciably increased when control of harmful insects was obtained by the use of insecticides; (*b*) honeybee pollinator activity was most intense within a few hundred yards of the apiary; (*c*) seed yield varied directly with pollinator activity; (*d*) gathering of red clover pollen by honeybees was maintained at a high level, and (*e*) the insecticides used did not cause pollinator mortality. In the present paper further data on insect control, pollination and nectar secretion are reported.

CONTROL OF INJURIOUS INSECTS

Experimental Procedure

A 24-acre portion of a 42-acre field of "Ottawa" red clover was chosen as the experimental area. Adverse weather conditions delayed the removal of the first growth until July 10. The area was divided into 24 one-acre plots, thus permitting the use of four replicates of each of the following treatments:

1—DDT applied just prior to bloom.
2—DDT applied at full bloom.
3—DDT plus Toxaphene applied just prior to bloom.
4—DDT plus Toxaphene applied at full bloom.
5—DDT applied just prior to bloom and Toxaphene at full bloom.
6—*Control*—No insecticides applied.

[1] Joint contribution from the Divisions of Apiculture and Forage Crops, Experimental Farms Service, and Division of Entomology, Science Service, Canada Department of Agriculture, Ottawa, Canada.

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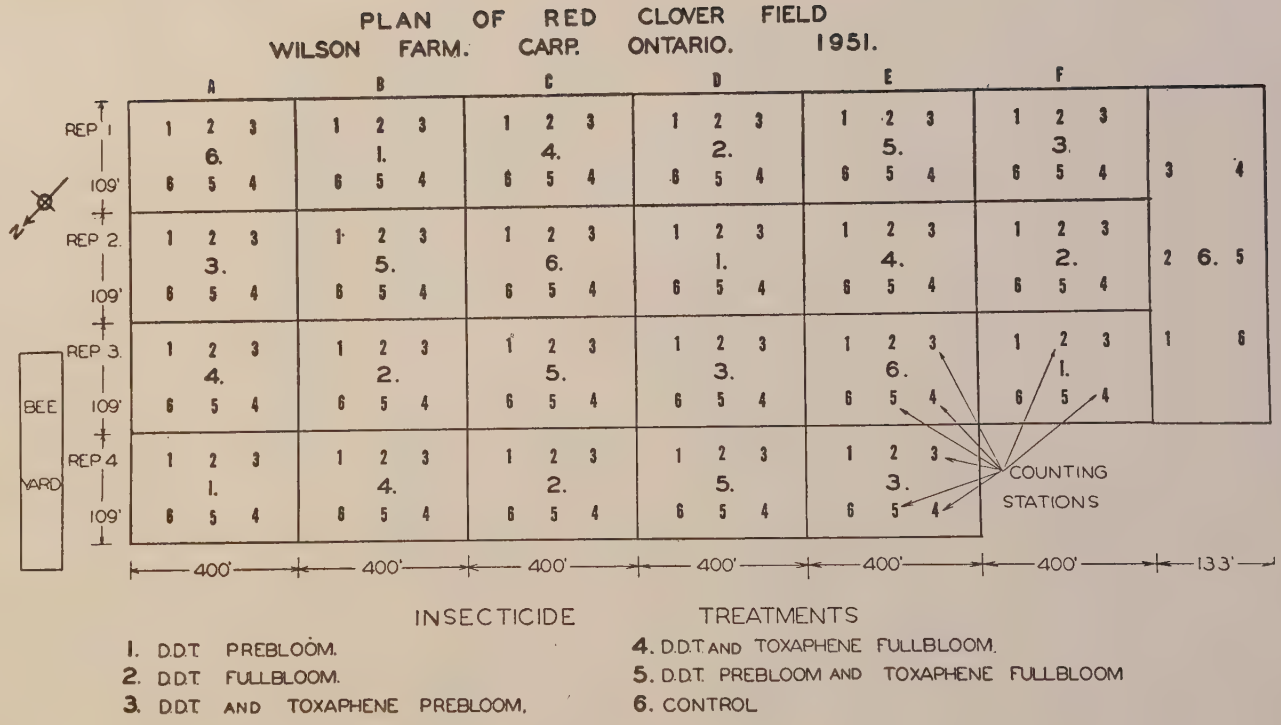
PLAN OF RED CLOVER FIELD
WILSON FARM. CARP. ONTARIO. 1951.
A
B
C
D
E
F
REP 1
REP 2.
REP 3.
REP 4
109'
BEE YARD
400'
133'
COUNTING STATIONS
INSECTICIDE TREATMENTS
1. D.D.T PREBLOOM.
2. D.D.T FULLBLOOM.
3. D.D.T AND TOXAPHENE PREBLOOM.
4. D.D.T AND TOXAPHENE FULLBLOOM.
5. D.D.T PREBLOOM AND TOXAPHENE FULLBLOOM
6. CONTROL

FIGURE 1.

Insecticides were applied as sprays at a pressure of 125 p.s.i., using approximately 50 gallons of water per acre. All chemicals were applied at the rate of 1.5 lb. of active ingredient per acre, whether used singly or in combination. Pre-bloom applications were made on August 3 and full-bloom applications on August 29.

Dead-bee traps were placed on five additional colonies to determine if the insecticide treatments caused excessive pollinator mortality.

Seed yields were obtained by extensive sampling; twenty 1-square-yard samples were drawn from each of the treatments in all replicates.

Experimental Results

The seed yields are recorded in Table 1.

TABLE 1.—YIELDS OF CLEAN SEED OF RED CLOVER FROM AREAS SUBJECTED TO INSECT CONTROL TREATMENTS

Treatments	Mean yield lb. per acre
1. DDT − (pre-bloom)	19
2. DDT − (full bloom)	20
3. DDT + Toxaphene (pre-bloom)	30
4. DDT + Toxaphene (full bloom)	27
5. DDT (pre-bloom) + Toxaphene (full bloom)	24
6. Control	22
General mean	24
L.S.D.	None

There were no significant differences apparent for any of the treatments. Yields were extremely low throughout the experimental area.

Systematic sweeps, made before and after application, indicated that the insecticides gave a measure of control of the pea aphid and tarnished plant bug. In Table 2, data are recorded on the infestation of the red clover seed midge in the experimental field.

It will be observed that the infestation ranged from 67.7 per cent for the DDT (pre-bloom) treatment to 71.1 per cent on the control area. Over the whole experimental area approximately 70 per cent of the red clover heads were destroyed or damaged in some degree.

Honeybee Mortality

Counts made in dead-bee traps, over a 10-day period, between August 17 and 30, showed the daily mean death rates from five colonies to be: 13.7, 48.9, 18.9, 112.5 and 33.2, respectively. The death rate in the colony which showed a mean of 112.5 may be attributed to robbing rather

TABLE 2.—INFESTATION OF RED CLOVER BY THE RED CLOVER SEED MIDGE

Treatment	Heads of red clover						
	Number per 4 square feet	Number infested	Per cent infested	Number grade 1*	Number grade 2*	Per cent grade 2	Per cent control
1. DDT, pre-bloom	229	155	67.7	79	76	33.2	0
2. DDT, Toxaphene, pre-bloom	232	163	70.2	76	87	37.5	0
3. Check	232	165	71.1	90	75	32.3	—

* Grade 1 = 1 – 50 per cent of florets infested.
* Grade 2 = 51 – 100 per cent of florets infested.

than the insecticide applied. Examination did not reveal any disturbance of the brood-rearing cycle within the colonies.

Discussion

It is probable that the heavy infestation of the red clover seed midge can be attributed, in some measure, to the late cutting (July 10) of the first growth. However, a survey of fields in the same area, from which the first growth had been removed at what was considered to be a satisfactory time for subsequent seed setting, revealed extensive damage from the midge. The low seed yields obtained can be attributed to both the late cutting of the first growth and the incidence of the seed midge.

POLLINATION

Experimental Procedure

Eighty colonies of honeybees were moved into the 42-acre field on August 14 and removed on September 14. The bees were located at the north end of the field on which insecticides were applied and on which pollinator counts were made. With the exception of three colonies, located approximately one-half mile from the south end of the field, no other honeybee colonies were known to be within pollinating distance of the experimental area.

Six observational areas were established at 400-foot intervals from the apiary as outlined in Figure 1.

Each observational area was subdivided into four replicates, on each of which were located six pollinator-counting plots. The counting plots, two square yards in area, were located at 100-foot longitudinal intervals from the apiary. Half-minute counts were made at each counting plot, beginning at 10.30 a.m., 1.30 p.m., and 3.30 p.m.

Pollen traps were placed at the entrances of fifteen colonies to determine the amounts and kinds of pollen being gathered. Pollen collections were made on 8 days during the period of August 24 to September 12. The pollen was air-dried and weighed. A one-gram sample of each lot, containing approximately 200 pellets, was set aside for analysis. The pollen

TABLE 3.—VARIATION IN POLLINATOR ACTIVITY AS TO DAYS AND TIME OF DAY AS INDICATED BY MEAN COUNTS

Item	Mean counts on 2-square yard areas based on 288 observations							
	Range of pollinator activity		Pollinators					
			Honeybees		Bumblebees		Honeybees	Bumblebees
	Honeybees	Bumblebees	A.M.	P.M.	A.M.	P.M.		
Aug. 17 to Sept. 12 (12 count days)	2.88 to 16.42	2.17 to 4.54	—	—	—	—	—	—
Time of day	—	—	4.76	9.82	2.06	4.64	—	—
General mean	—	—	—	—	—	—	6.56	2.99
L.S.D. (P = 0.05)	2.00	0.83	2.31	—	0.76	—	—	—

pellets were first separated on the basis of colour (1), (3), and identification was confirmed by microscopic examination of pollen grains from these pellets.

Experimental Results

1. Pollinator Activity

It was possible, by the use of cumulative totals, to arrange the data so as to establish the over-all variations of pollinator activity, (*a*) between days; (*b*) between morning and afternoon, i.e. 10.30 a.m., and 1.30 p.m.–3.30 p.m., and (*c*) with distance from the apiary. Table 3 indicates the variations in pollinator activity between honeybees and bumblebees.

These data show that, as in 1950, honeybee and bumblebee activity varied greatly from day to day, and that pollinator activity was much greater in the afternoon than in the morning.

2. Placement of Colonies

The effect of colony placement on pollinator effectiveness was measured. Table 4 indicates the population of honeybees and bumblebees at various distances from the apiary.

TABLE 4.—POPULATION OF HONEYBEES AND BUMBLEBEES AT MEASURED DISTANCES FROM APIARY

Distance from apiary	Honeybees	Bumblebees
	Mean per replicate	Mean per replicate
A (0 – 400′)	117.13	44.75
B (400 – 800′)	113.13	39.00
C (800 – 1200′)	76.13	34.50
D (1200 – 1600′)	69.88	31.75
E (1600 – 2000′)	43.75	33.75
F (2000 – 2400′)	52.50	31.75
General mean	78.75	35.92
L.S.D. (P = 0.05)	40.62	None

The data recorded in Table 4 show that the honeybee populations varied from 117 per replicate at the observation point "A" to 52 per replicate at observation point "F". There were significant variations with distance from the apiary. The bumblebees were fairly constant in numbers and were dispersed relatively uniformly throughout the field.

Table 5 shows the seed yield and pollinator counts at 100-foot intervals from the colonies.

TABLE 5.—INFLUENCE OF COLONY PLACEMENT ON SEED YIELD AND HONEYBEE POLLINATOR ACTIVITY

Station designation	Distance from apiary in feet	Yield of clean seed per acre in pounds (means of 8 obs.)	Honeybees per 2 sq. yd. area (means of 288 obs.)
A	(130′)	62.43	16.5
	(230′)	44.14	17.2
	(330′)	46.67	16.3
B	(530′)	42.85	16.5
	(630′)	41.97	16.3
	(730′)	38.25	15.0
C	(930′)	30.85	10.1
	(1030′)	27.72	11.5
	(1130′)	20.02	7.5
D	(1330′)	15.52	9.2
	(1430′)	15.23	10.0
	(1530′)	11.03	10.0
E	(1730′)	8.10	4.6
	(1830′)	7.08	4.8
	(1930′)	10.59	3.3
F	(2130′)	11.53	5.7
	(2230′)	8.48	5.2
	(2330′)	10.66	4.8

While the seed yields are extremely low, the data indicate a similar trend to that obtained in 1950. The yields from the area in close proximity to the apiary were five times greater than those from the location farthest removed from the apiary. Simple correlation values were obtained as follows:

	r Value obtained	r Value required
Honeybees and distance	0.963	0.443
Honeybees and seed yield	0.920	0.443
Distance and seed yield	0.934	0.443

3. Pollen Collections

The data recorded in Table 6 show that the quantity and type of pollen gathered varied widely from day to day. The amount of red clover pollen gathered increased progressively from August 24 to September 12. The heavy collection of red clover pollen in September was made at a time when relatively little pollen from other species was available. The total daily honeybee pollinator counts on red clover on each of the two days in

TABLE 6.—POLLEN COLLECTED FROM VARIOUS PLANT SPECIES BY FIFTEEN COLONIES ON 6 DAYS IN AUGUST AND 2 DAYS IN SEPTEMBER—GRAMS

Date, 1951	Red clover	Buckwheat	Goldenrod	White clover	Others	Total	Per cent red clover
August 24	38	588	381	25	23	1055	4
August 25	67	381	183	57	17	705	10
August 27	166	311	306	56	31	870	19
August 28	184	314	268	65	37	868	21
August 29	227	301	237	59	25	849	27
August 30	286	202	144	103	17	752	38
September 10	122	1	15	23	4	165	74
September 12	228	1	11	29	5	274	83
Total	1318	2099	1545	417	159	5538	24

TABLE 7.—WEIGHT—IN GRAMS—OF VARIOUS POLLENS GATHERED IN 6 DAYS IN AUGUST AND 2 DAYS IN SEPTEMBER, 1951, ARRANGED BY COLONIES

Colony No.	Red clover	Buckwheat	Goldenrod	White clover	Others	Total	Per cent red clover
234	202	30	35	11	8	286	71
138	179	162	152	35	20	548	33
1	138	135	258	64	16	621	22
175	109	251	94	31	2	487	22
131	109	149	83	19	12	372	29
142	94	120	105	23	3	345	27
133	92	267	180	78	26	643	14
121	73	105	74	14	20	286	26
148	70	235	179	63	26	553	13
232	61	225	130	27	16	459	13
114	58	185	91	33	5	372	16
130	47	36	30	6	1	120	39
185	38	85	58	4	3	178	21
143	31	92	41	5	1	180	17
146	7	22	25	4	—	58	11
Total	1318	2099	1545	417	159	5538	24

September were more than double those on any single day in August. On the basis of all pollen gathered, red clover was third in order of importance with 23.9 per cent of the total weight.

Colony preference for various types of pollen is indicated in the data given in Table 7. It will be observed that one colony collected only 11.0 per cent red clover pollen while another collected 71.0 per cent.

The areas surrounding the experimental field comprised a rather extensive acreage of competing plants in bloom at the time when the honeybees were moved into the area. Cursory examination of honeybee present on these competing crops, within flying distance from the apiary, indicated that numbers ranged from 3 to 4 per two-square yard area per half-minute in alfalfa to as high as 10 to 12 on buckwheat. As these crops matured higher collections of red clover pollen were recorded.

TABLE 8.—NECTAR READINGS IN RED CLOVER FOR A 9-DAY PERIOD. (MEANS OF 4 OBSERVATIONS)

Day	Station A (400′)		Station B (800′)		Station C (1200′)		Station D (1600′)		Station E (2000′)		Station F (2400′)		Means of stations	
	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.	Conc.	Vol.
	Per cent solids	μls. per floret	Per cent solids	μls. per floret	Per cent solids	μls. per floret	Per cent solids	μls. per floret	Per cent solids	μls. per floret	Per cent solids	μls. per floret	Per cent solids	μls. per floret
Aug. 20	22.0	0.71	22.3	0.62	22.0	0.57	20.0	0.63	22.3	0.74	19.3	0.78	21.3	0.67
Aug. 23	20.5	0.48	18.8	0.48	18.0	0.57	21.0	0.55	17.0	0.78	18.5	0.59	19.0	0.57
Aug. 24	26.8	0.49	28.3	0.90	23.0	0.76	24.5	0.86	26.3	0.55	25.5	0.75	25.7	0.72
Aug. 25	25.8	0.93	26.5	0.90	25.5	0.75	25.5	0.93	25.5	0.85	23.8	0.92	25.4	0.88
Aug. 28	32.5	0.74	30.8	0.58	29.8	0.93	30.3	0.87	30.3	0.69	31.5	0.78	31.0	0.76
Aug. 29	35.3	0.81	32.8	0.76	32.0	0.58	30.5	0.81	30.5	0.87	32.5	0.86	32.5	0.78
Aug. 30	36.0	0.99	29.3	1.06	31.0	1.00	28.0	0.87	28.0	1.25	29.3	1.03	30.0	1.03
Sept. 10	43.0	0.50	40.5	0.48	39.8	0.44	41.0	0.37	41.0	0.49	33.5	0.50	39.0	0.46
Sept. 12	32.8	0.50	33.5	0.66	33.8	0.59	34.3	0.67	34.3	0.64	33.8	0.53	33.5	0.60
General mean	30.5	0.68	29.2	0.71	28.3	0.69	28.3	0.73	27.7	0.76	27.5	0.75	28.6	0.72

L.S.D. (P = 0.05). Days and concentration of nectar – 2.0
Days and volume of nectar – 0.3
Distance and concentration of nectar – 1.6
Distance and volume of nectar – None

Discussion

The data obtained in this study indicate that maximum activity of honeybees and bumblebees occurs in the afternoon. It is evident also that many factors influence the work of the pollinators since very wide variations occur even though weather conditions seem favourable for flight. It was observed, as in 1950, that the periods of greatest activity of the bumblebees do not always coincide with those of the honeybees. While in the previous year the honeybees and bumblebees were present in the ratio of 8 : 1, the ratio was 2.2 : 1 under the conditions of this experiment.

The data presented here, and previously, indicate that maximum pollinator efficiency can only be obtained if the colonies are dispersed throughout large fields. The above data are in accordance with the results obtained by Park and Walstrom (5) and Firsov and Kamaeva (2) Pollinator efficiency will be conditioned by such factors as size of the field, the types of vegetation on adjacent areas, and weather conditions prevailing during the flowering period.

The honeybee will visit red clover to gather pollen in quantity, particularly when that of competing crops is not available.

While the data presented would seem to show colony preference for different types of pollen, substantiating data will be required before definite conclusions can be drawn.

NECTAR SECRETION

Experimental Procedure

Four uniform samples of heads of the same maturity were drawn from each station on each replicate on the afternoons of 9 separate days. Nectar was extracted from these heads by the centrifuge method used by Swanson and Shuel (6). Determination of total solids was made on a Zeiss refractometer. Floret counts were made on each head to permit the expression of volume on a per floret basis. The average number of florets was one hundred per head based on 3,200 counts.

Daily nectar volume and concentration at the various stations are shown in Table 8.

Experimental Results

These data indicate that significant variations occurred in nectar volume and concentration between days. While concentration varied significantly with distance, volume did not. Relatively high volume readings were obtained as compared to those in 1950. On the other hand, per cent solids were low. Per cent solids in the nectar varied inversely with the volume.

Discussion

The 1950 studies on nectar secretion gave conclusive data. It was found that the volume and concentration of nectar varied significantly between days, while during the day the volume was significantly higher in

the morning, and the concentration higher in the afternoon. The 1951 data, drawn from observations made at intervals during the blooming period, conform to these findings. The significance of these nectar variations, as related to pollinator activity, has not been determined.

ACKNOWLEDGMENTS

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BIOTIC FACTORS OF CONTROL OF THE EUROPEAN FIR BUDWORM, *CHORISTONEURA MURINANA* (HBN.) (N. COMB.), IN EUROPE[1]

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ABSTRACT

Choristoneura murinana (Hbn.), a budworm attacking fir, *Abies alba* Mill. in Europe, and very closely related to the spruce budworm, *Choristoneura fumiferana* (Clem.), was studied to obtain agents of biological control to aid in the fight against the spruce budworm in Canada. Search in the areas of previous outbreak of *C. murinana* led to the discovery of a large population of economic importance in the Vosges Mountains of France and an endemic population of no economic significance in the Black Forest of Germany. Both of these populations were studied to determine the extent of mortality suffered by the insect and what portion of this could be attributed to biotic factors of control. Although both the larvae and pupae of the European budworm were attacked by a complex of insect parasites, these were of secondary importance to a virus disease in reducing the population. The virus disease was distinguished by the presence in the cytoplasm of the infected cells of myriads of small, dense capsules. The organism has been given the name *Bergoldia calypta* Steinhaus.

INTRODUCTION

During 1947 and 1948 the author directed investigations on a number of European tortricids related to the spruce budworm, *Choristoneura fumiferana* (Clem.), primarily to obtain agents of biological control such as insect parasites, predators, and diseases. Those European tortricids considered most likely to yield useful biological enemies by virtue of their close relationship to the spruce budworm or their similarity in habitat and general ecology were the following:—

Choristoneura murinana (Hbn.)	on fir, *Abies alba* Mill.
Cacoecia rosana L.	on deciduous trees
Cacoecia histrionana Froel.	on spruce, *Picea abies* (L.) Karst.
Semasia rufimitrana H.S.	on fir, *Abies alba* Mill.
Semasia diniana Gu.	on larch, *Larix decidua* Mill.

The most intensive study was made on *Choristoneura murinana* because of its very close relationship to the spruce budworm not only in taxonomic position but also in similarity of habits. In the past it has been assigned to a number of genera, including *Tortrix* and *Cacoecia*, but according to a forthcoming revision of the tortricids by N. S. Obraztsov (in litt.), American Museum of Natural History, New York, it belongs to the genus *Choristoneura* Ld.

LIFE HISTORY AND HABITS

The moths of *C. murinana* occur in late June and the females deposit their eggs in a double row on the needles in the middle and upper crowns of mature white fir. Immediately after hatching, the larvae seek hibernating

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places between the bud scales or in cracks in the bark and spin fine white hibernacula within which they moult and pass the rest of the summer and the following winter in diapause in the second instar. In the spring, as the fir buds begin to burst, the larvae emerge from their hibernacula and begin to feed on the buds. There are 6 larval instars and the greatest volume of feeding occurs during the final instar. Pupation begins about the end of May; most of the pupae are formed on the ground. The adults emerge about 10 days later.

There are three periods of dispersal. The first occurs when the young larvae seek hibernating places and the second when they begin to attack the buds in the spring. At these times they spin long threads and may be carried for some distance by wind. A third dispersal takes place in the third or fourth instars when, having destroyed the first buds, they move upwards in search of more food.

The damage sustained by the fir trees is due to feeding on the new needles of the current year or, more rarely, on the epidermis and cambium of young shoots. Needles of previous years are never eaten. Only in areas of extremely heavy population of two or three years' duration does the insect cause death of the trees directly, but even in moderate attacks it may reduce the amount of wood laid down in annual rings. Furthermore, heavy attacks are frequently followed by an increase in population of the bark beetle *Ips curvidens* Germ., which may destroy many of the weakened trees. Infestations of *C. murinana* may be associated with populations of another budworm of somewhat similar habits, *Semasia rufimitrana*, or with *Epiblema nigricana*, which destroys the small buds formed for the following year. Such combinations make tree survival less likely. Generally, damage by *C. murinana* occurs only in stands where a high percentage of fir trees are mature or nearly so. When blown to young fir or spruce by the wind, the larvae can readily feed and complete development on these trees, but eggs are never laid on them. Thus, isolated stands of spruce or young fir escape attack, but these trees, existing under a cover of mature fir, may be very heavily attacked by larvae falling out of the high crowns.

PREVIOUS AREAS OF INFESTATION

The following information is based on surveys of the literature by Eidmann and Komárek, information supplied by the forest services of France, Germany, and Czechoslovakia, and ground surveys by the author and his assistants. References in support of the data may be obtained from the author.

The first damage by *C. murinana* was recorded in 1805 in the German Thüringerwald. From 1852 to 1862 a large outbreak occurred in the forests of northwestern Bohemia and further damage was recorded in Bohemia about 1866. Between 1867 and 1877 great damage was caused in Moravia and this infestation spread southward into Austria, where it reached its peak between 1872 and 1882. From 1888 to 1893 a series of heavy outbreaks occurred in the Schwarzwald of Germany and in Switzerland in the plain between the Alps and Jura Mountains, but the insect had been recorded from the Schwarzwald and from Saxony before this

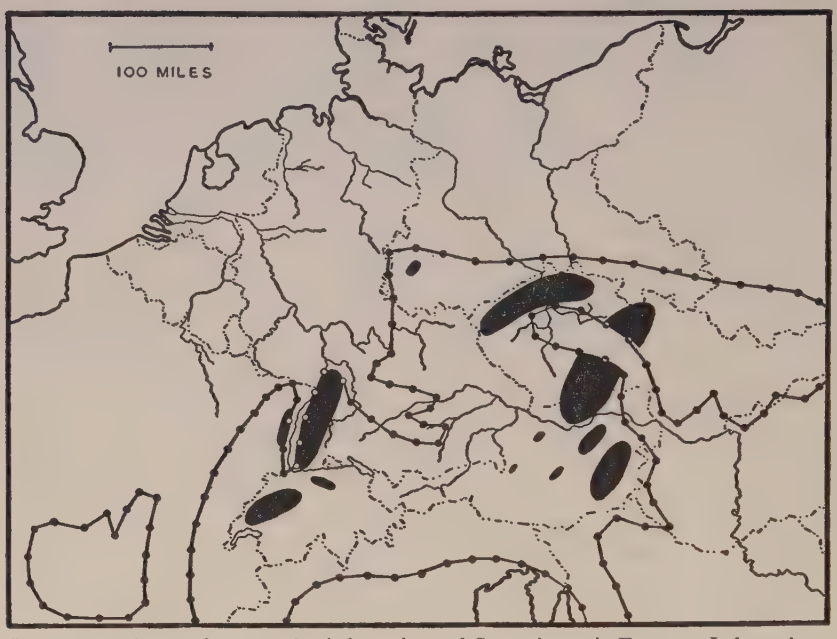

FIGURE 1. Areas of past major infestations of *C. murinana* in Europe. Infestations are shown in black and the original distribution of white fir is bounded by the line of connected dots.

major population increase. Between 1923 and 1928 some damage occurred in the Vosges Mountains of France, across the Rhine from the Schwarzwald. This appeared to be the forerunner of a series of infestations stretching across Europe, for the Schwarzwald again suffered heavily between 1931 and 1941. The insect spread eastward into Bavaria, where little damage occurred, but it caused great economic loss in Austria between 1931 and 1934 and in the forests of Bohemia and Moravia from 1933 to 1943. This cycle seems to have been brought to a close by the end of the last outbreak in Moravia, at Plumlov, in 1946. After the latest epidemic in the Schwarzwald, in 1941, small populations remained in the southern districts until about 1944; intensive search in 1947 yeilded a few specimens at Lörrach, Staufen, and Sulzburg, and revealed an endemic population of low magnitude at Gernsbach. In 1947 a heavy outbreak of limited extent was found in the Vosges Mountains of France near Ribeauville.

The major localities of occurrence are shown on the map (Figure 1), along with the original distribution of the chief food plant, white fir, *Abies alba*. In Europe at present, most of the fir stands have been artificially planted and the range of fir is considerably greater than shown. In addition, many of the fir stands in the original zone have been planted on land originally occupied by a beech-oak association. This practice probably makes the trees more susceptible to attack.

C. murinana is not recorded from Finland or Scandinavia. There is one record of collection from northern Italy, but no others from the whole of the Mediterranean area. There are no records of its presence in Hungary, Rumania, or the Balkan countries. There are no references in the literature

to its occurrence in the Baltic areas or Russia. There is evidence of one outbreak in Poland. One doubtful record exists from Holland. The insect is not recorded from northern Germany or Belgium or from France except in the Vosges area.

THE VOSGES INFESTATION

The infestation of *C. murinana* in the Vosges Mountains near Ribeauville was discovered in 1947. It consisted of three areas known as Kaysersberg, Pepiniere, and Tannenkirch, which were isolated one from another by high hills or by disappearance of the fir in favour of deciduous trees. The areas contained stands of fir lightly mixed with beech, oak, and spruce, and with some pine on high grounds. Except for the Kaysersberg area, the stands had an undercover of young fir trees.

Needle examination indicated that a small population of *C. murinana* had existed in 1946. In 1947, a heavy concentration of larvae caused great damage in all three areas. In each area, at the focus of the infestation, trees lost up to 90 per cent of the current season's needles, loss being reduced in intensity toward the upper limit of infestation at 600 metres above sea level. In general, it was estimated that a 50 per cent needle loss was sustained by the trees. But, as shown later, oviposition was light in comparison with the density of the larval population. In 1948, the infestation was much reduced in intensity. At the foci, some trees lost as much as 50 per cent of the current needles, but the over-all loss was only about 10 per cent. In 1948, observations were made from April to July to cover the complete active period of the insect. Periodic collections were made and the insects reared in a small field laboratory to obtain data on survival, parasitism, and disease.

Phenology of the 1948 Infestation and Duration of Life Stages and Instars

Figure 2 shows progressively the changes in the percentage of the population present in the various life stages and larval instars during the summer of 1948. The data were obtained by periodically sampling the population and expressing the number of insects in each instar as a percentage of the total population. The mean length of each stage is obtained by measuring the time between the crossings of the curves for the stages concerned. The duration of any one stage was dependent partly upon inherent characteristics and partly upon temperature, as shown by comparison with a similar graph given by Franz (3) for the Schwarzwald infestation of 1939. In the Vosges in 1948, the feeding period of instar II lasted only 10 days and instar III 6 days, as compared with 22 and 13 days in the Schwarzwald in 1939, during a period of cold, wet weather. Instar IV lasted 2 days and instar V 4 days in the Vosges as compared with 4 days each in the Schwarzwald. In the Vosges, instar VI was lengthened by cold, rainy weather to 20 days from 15 days recorded in the Schwarzwald. The pupal stage lasted 11 days in the Vosges, 12 in the Schwarzwald. In the Vosges, adults were observed between June 9 and July 4 and oviposition occurred during the later half of this period. Hatching took place during the first two weeks of July, and by July 20 the new population of larvae had passed through the first instar and was in the second instar in a dormant condition.

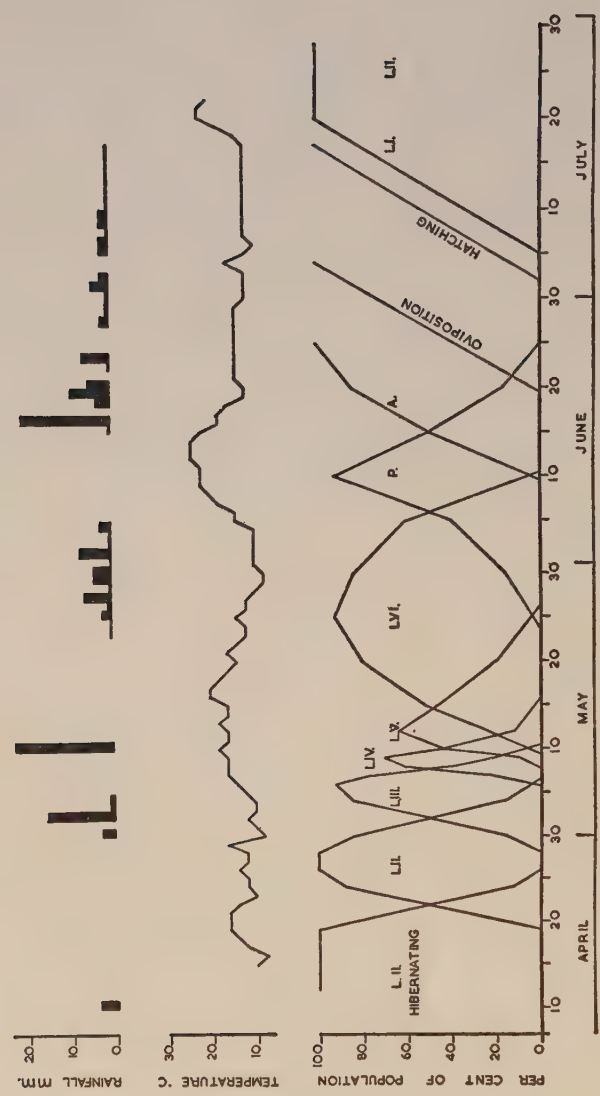

FIGURE 2. Progressive changes in the percentages of the population of *C. murinana* in various life stages and larval instars in the Vosges in 1948. L. II to L. VI, larval instars; P, pupa; A, adult. The temperature curve records mean daily readings at 3 m. above ground and 300 m. above sea level. The rainfall data were supplied by the meteorological station at Ribeauville.

Mortality

From field observations and surveys, from specially reared field samples, and from records of general laboratory rearing, an attempt has been made to estimate the mortality occurring in the population throughout the season and to determine the principal factors responsible.

Egg.—A small number of egg masses did not hatch. These had probably been laid by unfertilized females. Of 400 masses collected in the forest in each of the three areas, an average of 3 per cent showed no sign of development. About the same percentage of laboratory-deposited masses failed to hatch.

In any given egg mass, some of the eggs failed to hatch, the embryos developing up to a point where the head capsule could be seen. There was considerable variation in mortality between the three areas (Table 1). The cause of this variation is unknown but it may be associated with the virus disease, which was most prevalent at Tannenkirch. For the whole area it was estimated that 10 per cent of the eggs failed to hatch.

TABLE 1.—PERCENTAGES OF UNHATCHED EGGS OF *C. murinana* FROM EACH OF THREE AREAS IN THE VOSGES INFESTATION. IN ALL, 6 SAMPLES OF 500 EGGS EACH

	Kaysersberg	Pepiniere	Tannenkirch
Field-collected	3.0	9.2	16.4
Laboratory-deposited	2.0	11.4	14.4

Instar I.—Newly hatched larvae sometimes died before building hibernacula. Mortality at this stage averaged 4 per cent, and was independent of whether the eggs were deposited in the laboratory or collected in the field in any of the three areas.

Instar II, Hibernation.—In January, 1948, a collection was made of overwintering larvae in the Pepiniere area. About 35 per cent of these were dead when found. Summer temperatures during 1947 were much higher than normal whereas the winter of 1947-48 was comparatively mild.

At the end of the 1948 season about 300 newly hatched larvae from each of the three collection areas were allowed to form hibernacula in small vials, and were held at outside temperatures. In November percentage mortalities were 27.4 for Tannenkirch, 24.1 for Pepiniere, and 27.1 for Kaysersberg, the mean being 26.2. In 1948, summer temperatures were relatively cool and fall temperatures about normal. This indicates that most of the mortality occurs before the onset of cold weather. On the basis of the above observations, mortality during hibernation for each year, 1947 and 1948, was estimated to be 35 per cent.

Instar II, Spring Emergence.—When the larvae emerge in the spring, they spin quantities of web, upon which they drop and hang until they reach the middle and lower branches of the tree or are blown away by the wind. The larvae are strongly phototactic and climb readily; young larvae are resistant to starvation. If an undercover of small fir or spruce is present beneath the mature trees, the larvae readily become established

on these, and mortality during this early dispersal is small. If the undercover of young evergreens is light or lacking, mortality during the first spring dispersal may be heavy.

Franz (3) observed that low temperature and rain during the period of spring emergence prevented some larvae from becoming established in new buds. He estimated that mortality due to unfavourable climatic factors at this time reached 40 per cent. In the Vosges, in the spring of both 1947 and 1948, climatic conditions were very favourable to establishment and were not responsible for appreciable mortality. No estimate of mortality during emergence and establishment was made.

Instars III-VI.—During 1948 the larvae collected in the field were reared in the laboratory. Records were kept of the number of larvae that developed to pupae and of the number that died. The dead were classified into groups by visual inspection followed by microscopic examination with the dark-field microscope. Later, the results were checked by examination of samples with the electron microscope, through the courtesy of the Forest Insect Laboratory, Sault Ste. Marie, Ont. Dead larvae were classified as belonging to one of the following groups:—

Virus: This group contained insects showing typical symptoms of the virus disease.

Fungus: This group was characterized by a rosy colouration of the insects after death. Later the body became firm and then hard and the colour changed to rusty brown. Microscopic examination showed the presence of a mycelium within the insect body but the fungus has not been identified. The fungus was believed to be secondary.

Starved: Both in the laboratory and in the field, many sixth-instar larvae died following symptoms that were reproduced experimentally by withholding food. The first sign of abnormality was a shortening of the body accompanied by a darkening of the colour. The colour change was a result of darkening of both the fat body and the integument and a folding of the latter. The gut was filled with yellowish-green liquid containing many particles in suspension; the gut walls became translucent. Normally the gut walls were opaque and enclosed a mass of leaf particles. In extreme cases, the larva resembled a small dumbell, 1 centimetre or less in length, bearing at one end the large, black head capsule and at the other the orange anal shield; death sometimes occurred before this extreme condition was reached. If food was withheld from mature larvae experimentally, they developed the above symptoms and died. Once the symptoms were readily recognizable, the process was irreversible: larvae did not readily eat and died even when supplied with food. However, starved larvae were present in laboratory rearing trays and in the field where food was plentiful. In the field these larvae were most frequently found on the ground, on log piles, or on small trees, where their presence would postulate a period of non-feeding during which symptoms could develop. It appears that the cause of death was not simply lack of food, but that some unbalance of the general physiology of the insect had occurred, the stimulus for which may have been a short period of starvation.

Parasitized: A small number of parasites emerged from larvae in rearing. The chief insect parasite was *Glypta murinanae* Bauer. A few round worms, belonging to the genus *Mermis*, emerged from the larvae. These died before they transformed to the adult form and could not be further identified; they have not been included in the mortality figures.

Prepupae: This group contained all insects that died during the prepupal stage or that did not form externally sound pupae. Microscopic examination failed to show the typical particles associated with the virus disease. Bacteria did not appear important as a cause of mortality. Death in this stage appeared to claim insects that were physiologically too weak to form healthy pupae.

Table 2 shows the percentage mortalities and survivals (expressed as healthy pupae) of larvae collected in the three field areas and reared in the laboratory, for three main periods of collection. Figure 2 shows that collections between May 8 and May 15 were made before the field population was predominantly in the sixth instar; those between May 16 and June 4 were made mainly from a population of sixth-instar larvae; those between June 5 and June 12 consisted of sixth-instar larvae and pupae.

TABLE 2.—PERCENTAGE MORTALITY AND SURVIVAL OF INSTARS V AND VI OF *C. murinana*. THE VOSGES INFESTATION, 1948

Dates and place of collections	Percentage mortality classified by group						Percentage survival (pupae)	Total number collected (100%)
	Virus	Fungus	Starved	Prepupae	Parasitized*	Total		
May 8–15—								
Tannenkirch	42.0	6.8	11.2	4.4	0.9 (0.5)	65.3	34.7	4,500
Kaysersberg	23.2	4.6	8.0	6.0	1.0 (0.7)	42.8	57.2	2,600
Pepiniere	21.0	1.2	5.0	5.7	0.7 (0.4)	33.6	66.4	930
May 16–June 4—								
Tannenkirch	25.6	3.7	19.3	12.4	0.7 (0.7)	61.7	38.3	39,800
Kaysersberg	20.2	3.2	13.7	14.3	1.6 (1.6)	53.0	47.0	17,800
Pepiniere	17.2	1.5	18.5	12.3	0.7 (0.6)	50.2	49.8	11,600
June 5–12—								
Tannenkirch	11.8	0.8	11.9	7.7	0.6 (0.6)	32.8	67.8	17,500
Pepiniere	7.3	0.1	3.0	11.1	0.6 (0.6)	22.1	77.9	1,520

* Figures in parentheses indicate parasitism by *Glypta murinanae*.

Table 2 shows that survival was higher in collections made before May 15 than those made between May 16 and June 4, when the larvae were chiefly in the sixth instar. This indicates that survival in the laboratory was at least as high as that in the field, and that mortality generally occurred in the sixth instar. Field observations confirmed that mortality was chiefly confined to the sixth instar, and to a much lesser extent to the fifth. Very little mortality occurred in the field in the early feeding instars, as was pointed out by Franz (3). Total mortality during the feeding instars III and IV was estimated at 5 per cent and included loss during the second dispersal.

In the sixth instar the larvae were more exposed to unfavourable conditions and more active and tended to leave their webs under the stimuli of wind and rain. Virus and starvation symptoms became evident. The larvae ate large volumes of food and were susceptible to short periods of starvation, such as might be encountered if they fell from the tree during agitation by windstorms and rain.

Survival was highest in the latest collections of the season, because many of the insects were collected as pupae or healthy prepupae. Larvae present at this time were in poor condition; some showed virus symptoms, a number showed starvation symptoms, and many of the remainder died as prepupae or were parasitized.

Table 2 shows that survival to the pupal stage was less for Tannenkirch than for the other two areas, and the increased mortality was due chiefly to the virus disease. An estimated field mortality of 50 per cent for the Vosges area as a whole during instars V and VI was due to the following factors: *virus*, 25 per cent; *fungus*, 3; *starvation*, 12; *prepupal deaths*, 9; and *parasites*, 1.

Pupa.—Pupae formed on the rearing trays were incubated to provide data on survival and on parasites that attack the larvae and emerge from the pupae. These had an adult emergence of 54 per cent; 1 per cent were parasitized by insects and the remainder died. Parasitism of pupae, collected in the field shortly before adult emergence, reached 5 per cent; this included insect parasites that made their attacks in the prepupal or pupal stages. On the basis of about 50,000 reared and field-collected pupae, it was estimated that 54 per cent survived to the adult stage, 5 per cent were parasitized, and 41 per cent died from unknown causes. Typical virus particles could not be demonstrated in dead pupae by either the light or the electron microscope. About 15 per cent of the dead pupae contained bacteria in numbers but it is unknown whether these were secondary invaders or primary in action.

Discussion of Factors Contributing to Reduction of the Population.—As mentioned above, in 1947 a very heavy population of larvae caused an estimated defoliation of 50 per cent in the Vosges infestation, but in 1948 defoliation was estimated at only 10 per cent. It was not possible to compare the density of the larval population in 1947 with that of 1948 except indirectly through comparison of the damage, but a comparison was made of the egg density in July 1947 and 1948. Five branches, one inch in diameter, were cut from the upper branches of each of 30 mature trees, selected as being attractive to ovipositing females. In 1948 these gave a

TABLE 3.—SUCCESSIVE REDUCTION OF A POPULATION OF 100 EGGS OF *C. murinana* OVER ONE COMPLETE GENERATION FROM FACTORS OPERATING AT VARIOUS LIFE STAGES IN THE VOSGES INFESTATION, 1947-48

Stage of development	Number of survivors from an original population of 100 eggs	Percentage reduction	Number by which population was reduced	Explanation of reduction
Egg	(100)	3	3.0	Egg masses from infertile females
Egg	97.0	10	9.7	Failure to hatch
Instar I	87.3	4	3.5	Death before diapause
Instar II (diapause)	83.8	35	29.3	Death during diapause and hibernation
Instar II (feeding)	54.5	?	?	Loss during spring establishment unknown
Instars III–IV	54.5	5	2.7	Death during early feeding and second dispersal
Instars V–VI	51.8	50	25.9	Killed by virus, 25%; fungus, 3%; parasites, 1%; starved, 12%; died as prepupae, 9%.
Pupa	25.9	46	11.9	Parasitized, 5%; unknown mortality, 41%
Adult	14.0	50	7.0	Sex ratio 1 : 1
Female	7.0	—	—	12.5 eggs laid per female
Egg (filial generation)	87.5	—	—	

mean of 7.8 egg masses per branch as compared with 9.8 masses in 1947, a reduction of about 20 per cent. If all of the factors contributing to mortality were accurately known along with the productivity of the female insect, it would be possible to construct a table to show how each factor operates to produce a reduction of 20 per cent in the population of eggs by acting in sequence through one complete generation. Although the mortality factors are not completely known, many of them have been estimated above and they have been compiled in Table 3 to show the reduction in a population, beginning with 100 eggs in 1947. After oviposition in 1948, the table shows a population of 88 eggs, a reduction of 12 per cent, which is in reasonably close agreement with the reduction found by egg surveys.

It is difficult to estimate the loss from climatic factors operating at critical periods in development, such as hibernation and spring emergence and establishment. Mortality during diapause was estimated at 35 per cent on the basis of two years' observations but neither of these seasons was severe. Franz (3) estimated a 40 per cent mortality of young larvae during emergence from their hibernacula in the spring of 1939 in cold, wet weather. In the Vosges in 1948, weather conditions were favourable and mortality during emergence and establishment was low and could not be assessed.

Determination of the productivity is also difficult because any space limitation, such as a cage, imposed on a female may affect her oviposition. In the Vosges, a total of 5000 caged females deposited an average of 12.5 eggs per female. This figure is much lower than that reported by Franz (3), but it is supported by field observations and dissections and is considered to be a reliable estimate of oviposition. A mated female contains about 25 mature eggs and others in various stages of maturity. Early deposition consists of the 25 eggs, which are mature shortly after copulation. Further maturation of the eggs and further oviposition are dependent upon the ability of the females to find and take food. Franz (3) mentioned that adults would take honey or sugar and that food was important in egg production. But in the Vosges, caged females did not take food and adults were never observed to feed in the field. This limited oviposition to those eggs that were already mature shortly after copulation, in this case to about 25 eggs. Both in experimental cages and in the field, over 30 per cent of females examined after mating showed a degradation of the ovaries and were without mature eggs. When allowance has been made for inability to find a mate and for mortality before oviposition, it is doubtful whether more than half of the females oviposit. Thus it was estimated that only half of the females laid 25 eggs, a mean of 12.5 eggs per female, a number which is in agreement with that found in experimental cages.

From an economic point of view, it is more interesting to examine the sequence of the factors beginning with the fifth larval instar in order to assess their importance in reducing the population during the stages when most of the damage is done. The data of Table 3 are rearranged in Table 4 to show the expected reduction in a population of 100 fifth-instar larvae beginning heavy feeding in the summer of 1948. If mortality in diapause and during spring establishment remained equal to that suffered in 1947-48,

TABLE 4.—ESTIMATED SUCESSIVE REDUCTION OF A POPULATION OF 100 FIFTH-INSTAR LARVAE OF *C. murinana* OVER ONE COMPLETE GENERATION FROM FACTORS OPERATING AT VARIOUS LIFE STAGES IN THE VOSGES INFESTATION, 1948-49

Stage of development	Number of survivors from an original population of 100 entering instar V	Percentage reduction	Number by which population was reduced	Explanation of reduction
Instars V–VI	(100)	50	50.0	Killed by virus, 25%; fungus, 3%; parasites, 1%; starved, 12%; died as prepupae, 9%
Pupa	50.0	46	23.0	Parasitized, 5%; unknown mortality, 41%
Adult	27.0	50	13.5	Sex ratio 1 : 1
Female	13.5	—	—	12.5 eggs laid per female
Egg	168.8	3	5.1	Egg masses from infertile females
Egg	163.7	10	16.4	Failure to hatch
Instar I	147.3	4	5.9	Death before diapause
Instar II (diapause)	141.4	35	49.5	Death during diapause and hibernation
Instar II (feeding)	91.9	?	?	Loss during spring establishment unknown
Instars III–IV	91.9	5	4.6	Death during early feeding and second dispersal
Instars V–VI (filial generation)	87.3	—	—	

only 87 larvae were expected to enter the fifth instar in the summer of 1949 and this reduction of 13 per cent was expected to result in correspondingly less damage to the forest. Louis Mesnil (in litt.), who made further studies in the Vosges in 1949, reported that damage was from 20 to 50 per cent less than in 1948. Table 4 clearly shows the importance of mortality during hibernation and spring establishment. For example, unfavourable spring weather, causing 40 per cent mortality of young larvae as found by Franz (3), might reduce the number of insects entering instar V to 52 per cent of the original population.

Tables 3 and 4 show that the greatest biotic factor of mortality was the virus disease. The importance of the disease can be readily demonstrated by following through Table 4, omitting virus mortality and assuming that mortality due to other factors remains constant. Then the end of one complete cycle shows 131 larvae entering instar V and ready to damage the forest, a population increase of 31 per cent rather than a decrease of 13 per cent.

Insect parasites were of little importance in reducing the population in the Vosges in 1948. But if mortality due to parasitism is omitted in Table 4, the end of one generation shows 97 mature larvae ready to damage the forest instead of 87. The possible benefits of parasites cannot be disregarded. In the small Schwarzwald population of 1939, at the tail of a long period of infestation, parasitism was the greatest single factor of mortality (3).

Franz (3) observed that predacious birds took a toll of the budworm population in the Schwarzwald in 1939. Bird life was rare in the Vosges; no birds were observed feeding on the larvae or pupae. Budworm larvae were free of insect predators.

Consideration may now be given to the factors involved in the decline of the Vosges population from 1947 to 1948. It has been pointed out above that comparison of twig damage and foresters' reports indicated that the population of fifth and sixth-instar larvae in 1947 was about five times that found in 1948. However, the number of egg masses in 1947 exceeded that in 1948 by only 20 per cent. This fact, coupled with foresters' observations of a relatively low level of moth flight, indicates that mortality in the sixth instar or pupa was much higher in 1947 than in 1948. Parasite attack in 1947 was probably even lower than in 1948. This leaves disease and starvation as the chief probable factors responsible for the drastic reduction in population.

Insect Parasites

Insect parasites did not play an important role in reducing the population of *C. murinana* in the Vosges. It may be assumed that some of the effects of parasitism have been masked by death of the host and the parasites from other causes. In other words, if the host did not die from other factors, a greater number would have died from parasite attack. This potential parasitism can be estimated by calculating percentage parasitism on the basis of surviving hosts. But even in the absence of death from other causes, larval parasitism would not have exceeded 2 per cent or pupal parasitism 10 per cent.

The parasites emerging from the larvae are listed below. Their relative importance is indicated by the number in parentheses:—

Ichneumonidae	Banchinae	*Glypta murinanae* Bauer	(1)
	Ophioninae	*Angitia vestigialis* (Ratz.)	(4)
	Tryphoninae	*Phytodietus segmentator* Grav.	(5)
Braconidae	Microgasterinae	*Apenteles dilectus* (Hal.)	(2)
		Apanteles ater (Ratz.)	(2)
	Blacinae	*Pygostolus sticticus* (F.)	(3)
	Euphorinae	*Meteorus ruficeps* (Nees)	(6)

The parasites emerging from the pupae were:—

Ichneumonidae	Pimplinae	*Itoplectis maculator* (F.)	(1)
		Apechthis resinator (Thunb.)	(2)
		Apechthis rufata (Gmel.)	(3)
		Pimpla turionellae (L.)	(6)
	Metopiinae	*Triclistus globulipes* (Desv.)	(4)
		Metacoelus femoralis (Geoffr.)	(8)
		Chorinaeus tricarinatus Holmgr.	(9)
	Ichneumoninae	*Phaeogenes scutellaris* Wesm.	(5)
	Ophioninae	*Agrypon variitarsum* (Wesm.)	(7)
Torymidae	Monodontomerinae	*Monodontomerus aereus* Wlkr.	(10)
Larvaevoridae		*Nemorilla maculosa* (Meig.)	(11)
		Bessa selecta (Meig.)	(12)
		Pseudoperichaeta insidiosa (R.D.)	(13)

THE SCHWARZWALD INFESTATION

The German Schwarzwald has suffered some of the heaviest outbreaks recorded for *C. murinana*, the most recent being between 1931 and 1941. Since then, the insect has maintained an endemic population without attracting much attention. In 1947, a questionnaire to 65 forest bureaux resulted in only three positive reports of the presence of the insect since 1941, and a scouting trip revealed its presence in three other areas. In 1948, collections of *C. murinana* larvae were made at Gernsbach in a stand of white fir about 60 years old. Since there was little undercover of young trees, about 300 trees were felled in order to make the collections, which were reared at the Kaiser Wilhelm Institut at Tübingen.

The population of *C. murinana* at Gernsbach differed from that of the Vosges in being small and causing little or no damage; it was probably of long duration, being the residue of the wave of infestation ending in 1941. It was not possible to compare all the mortality factors of this population with those of the Vosges, but laboratory rearing data are assembled in Tables 5 and 6.

Since early collections were not successfully reared in the laboratory and late collections included mature larvae and pupae, the most valid collections on which to base larval mortality were those made from May 7 to 18. The high parasitism of late collections was due to delayed pupation of parasitized larvae. Microscopic examination of samples indicated that 58 per cent of the dead larvae were infected with the virus; the fungus appeared in a few cases only. No evidence of the presence of pathogenic bacteria was obtained. Some larvae showed symptoms of starvation, but an accurate record was not kept of these. It was estimated that only 53 per cent of the larvae entering the feeding instars survived to the pupal

TABLE 5.—PERCENTAGE LARVAL MORTALITY AND SURVIVAL IN COLLECTIONS FROM GERNSBACH, 1948

Date collected	Instar or stage	Parasitized*	Dead from other causes	Survived to form pupae	Total (100%)
April 27–May 6	III–IV	2.7 (0.5)	77.8	19.5	870
May 7–18	V–VI	2.5 (1.6)	44.9	52.6	11,600
May 19–27	VI–pupa	4.5 (3.0)	27.9	67.6	3,650
May 28–June 5	Few VI pupa	17.4 (12.6)	14.7	67.9	1,000

* Figures in parentheses indicate parasitism by *Glypta murinanae*.

TABLE 6.—PERCENTAGE PUPAL MORTALITY AND SURVIVAL IN COLLECTIONS FROM GERNSBACH, 1948

Date collected	Instar or stage	Parasitized	Dead from other causes	Survived to form adults	Total (100%)
April 27–May 6	III–IV	0.0	17.1	82.9	170
May 7–18	V–VI	0.4	33.3	66.3	6,105
May 19–27	VI–pupa	8.7	43.1	48.2	2,469
May 28–June 5	Few VI pupa	24.0	27.0	49.0	679

stage; 26 per cent were killed by the virus disease, 3 per cent by parasite attack, and 19 per cent by unknown causes. Survival in the Schwarzwald was comparable to that in the Vosges.

Collections made between May 28 and June 5 gave the best estimate of pupal survival, because they consisted largely of field-collected pupae, exposed to parasite attack. It was estimated that 49 per cent of pupae produced adults, 24 per cent were parasitized, and 27 per cent died from unknown causes. Total pupal mortality in the Schwarzwald was comparable to that of the Vosges, but insect parasitism was more important, pupal parasitism in the Vosges being only 5 per cent. If the masking effect of unknown mortality is removed, by calculating parasitism on the number of surviving insects, the potential parasitism at Gernsbach would reach 33 per cent; pupal parasitism thus played an important part in the control of the host population.

The insect parasites emerging from the larvae are listed below. Their relative importance is indicated by the number in parentheses:—

Ichneumonidae	Banchinae	*Glypta murinanae* Bauer	(1)
	Ophioninae	*Angitia chrysosticta* (Gmel.)	(4)
		Angitia vestigialis (Ratz.)	(5)
		Angitia rufipes (Grav.)	(6)
	Pimplinae	*Ephialtes terebrans* (Ratz.)	(8)
		Pimpla sp.	(10)

	Tryphoninae	*Phytodietus segmentator* Grav.	(9)
	Cryptinae	*Hemiteles bicolorinus* Grav.	(hyper.)
		Hemiteles sp.	(hyper.)
	Mesochorinae	*Mesochorus* sp.	(hyper.)
Braconidae	Microgasterinae	*Apanteles dilectus* (Hal.)	(2)
		Apanteles ater (Ratz.)	(2)
	Euphorinae	*Meteorus ruficeps* (Nees)	(3)
	Blacinae	*Eubadizon extensor* (L.)	(7)
Elasmidae		*Elasmus albipennis* Thoms.	(hyper.)

The parasites emerging from the pupae were:—

Ichneumonidae	Pimplinae	*Itoplectis maculator* (F.)	(1)
		Pimpla turionellae (L.)	(3)
		Ephialtes inquisitor (Scop.)	(4)
		Ichneumon pictus Gmel.	(5)
	Ichneumoninae	*Phaeogenes scutellaris* Wesm.	(2)

OBSERVATIONS ON INDIVIDUAL PARASITE SPECIES

Investigations into the biology of the parasites were hampered by the low density of the parasite population. The following observations are presented as an introduction to further studies of budworm parasites, either in Canada or in Europe.

Larval Parasites

Glypta murinanae Bauer was the most important parasite emerging from the larval stage of *C. murinana*, and accounted for about half of all larval parasitism. It is apparently specific to its host, and was found by Franz (3) in the Schwarzwald in 1939. It was described by Bauer (1). The earliest collections produced specimens of the insect, so that attack must occur when the host larvae are very small, probably shortly after hatching. Hibernation takes place within the hibernating host larvae. *Glypta fumiferanae* (Vier.), a parasite of the budworm in Canada, has similar habits. Parasitized host larvae develop normally until the last instar, but the length of this instar is prolonged until most healthy larvae have pupated. Therefore, at the end of the collecting period, larval collections included a weighted percentage of parasitized larvae. The mature *G. murinanae* larva is about 11 mm. in length, white in colour with three pairs of ventral black thoracic spots, a black spot on the head, and a short tail. It lies loosely in the body cavity of the host, usually in a ventral position above the prolegs. On emerging from the mature host larva, it spins a thin, transparent cocoon about 12 mm. long between the needles of the fir tree. After several days, pupation occurs, and, in two to three weeks after cocoon formation, the adult emerges by gnawing an irregular hole in the flimsy cocoon.

Apanteles spp. were second in importance after *G. murinanae* in both areas studied. There appear to be at least two species attacking *C. murinana*, but the taxonomy of this group is so confused at present that they will be considered together. They attack the young larvae of *C. murinana* shortly after emergence or after construction of a hibernaculum; this habit is similar to that of *A. fumiferanae* Vier. on the budworm in Canada. The parasite overwinters within the host larva and continues its development the following spring. The host is retarded in development and only reaches the fifth instar, in which stage it is killed by the emerging parasite. The parasite larva spins a dense, white, oval cocoon about 3 mm. in length. The adult emerges by making a neat, circular cut in the anterior end and pushing off the so-formed door, which usually remains attached by a narrow hinge. The parasites emerged from the host from May 17 to 27 and the adults emerged from the cocoon about a week or ten days later. In the laboratory, males lived about 10 days and females about 20 days, some individuals living up to 40 days. Shortness of life may be a factor limiting the reproduction of the species. In 1948, hatching of the host eggs did not occur in quantity until the beginning of July. The parasite adults, therefore, had to remain from about June 7 to 30 before finding a stage of the host susceptible to parasitism. Under these conditions most of the parasite adults had died before they had an opportunity to parasitize *C. murinana* larvae.

Meteorus ruficeps (Nees) was third in importance among the larval parasites at Gernsbach, but was only rarely encountered in the Vosges. Two species of *Meteorus* have previously been recorded from *C. murinana*, but, unless there is some confusion in nomenclature, this is the first record of this species from this host. *M. ruficeps* attacks young larvae of *Cacoecia piceana* (L.) before hibernation of the host and overwinters in its host (4). It probably behaves in a similar manner when attacking *C. murinana*. Specimens were obtained from the earliest collections. The parasite larva emerges from the last larval instar of the host and forms a thin, translucent cocoon 5 to 6 mm. long. The emerging adult cuts a neat, circular door in the anterior end of this cocoon. Emergence from the host occurred between May 20 and June 10. The adults emerged from the cocoons about 10 days later. Both sexes have a long life in the laboratory, the males living about 20 days and the females about 40, with individuals of both sexes living over 50 days. Thus it is possible for the young host larvae of the next generation to be attacked in July.

Angitia chrysosticta (Gmel.), along with two other species, *A rufipes* (Grav.) and *A. vestigialis* (Ratz.), was next in importance. In the Vosges only *A. vestigialis* occurred. *A. chrysosticta* has been reported previously on *C. murinana*, and it is known to be parasitic on a large number of small Lepidoptera, chiefly tineids. The other species have not been recorded from *C. murinana* before. All three species apparently have similar habits and may be considered together. The parasites probably attack the young host larvae in the summer and overwinter within them, as the earliest spring collections showed parasitism by these insects. The parasite larva emerges from the last instar of the host and constructs a light-brown cocoon about 6 mm. long, overlaid with a light covering of coarser white

threads. These threads are denser at the equator of the cocoon and form a narrow white band. Parasite larvae emerged from the host between May 20 and June 10. About two weeks later, adults emerged from the cocoons. Adults lived for about two weeks in the laboratory.

Pygostolus stictícus (F.) and *Eubadizon extensor* (L.) were rare. These are first records on *C. murinana.*

Phytodietus segmentator Grav. was found only once both in the Vosges and at Gernsbach. This was the only external parasite found on *C. murinana.* The larva constructs a long, slim, yellowish-brown cocoon about one centimeter in length. The adult emerges by a large, irregular hole near the anterior end during the first week in July.

Ephialtes terebrans (Ratz.) occurred rarely at Gernsbach. It is the largest of the parasites of *C. murinana,* and the female has a very long ovipositor. The cocoon is very similar to that constructed by *Glypta murinanae.* The adults emerge by an irregular hole during the third week of June. It has not been previously recorded on *C. murinana.*

Hyperparasites

At Gernsbach four species of hyperparasites, represented only by single specimens, were reared from *C. murinana* larvae. It is not known what parasite species were acting as hosts. The parasites were *Hemiteles bicolorinus* Grav., *Hemiteles* sp., *Mesochorus* sp., and *Elasmus albipennis* Thoms.

Pupal Parasites

The pupal parasites all attack the pupae or prepupae or almost mature, last-instar larvae. Except for the tachinids, which construct puparia outside the host, they utilize the protective covering offered by the host pupal skin and make no external cocoons. They begin to emerge from the pupa after the peak of host emergence has passed; none enter diapause within the host cocoon. Thus the adults emerging in June are faced with the alternatives of finding alternate hosts for a second generation, or of passing the remainder of the summer and the following winter and spring as adults, before the next generation of the budworm has reached a stage susceptible to parasitism. The duration of this period is almost one year. In the laboratory, individuals of several species lived for over 50 days, but the mean was only about a month. It seems likely that the maintenance of the population, at least for most species, is dependent upon the successful search for alternate hosts in July or August. Most of the parasites are known to be polyphagous and could readily maintain themselves on a variety of hosts. In the Gernsbach and Vosges areas, however, repeated searches failed to reveal the presence of populations of hosts that would serve to maintain the parasite density. Herein lies the chief weakness of the parasites as factors in control of the budworm. Their numbers are limited by the presence of alternate hosts, are not dependent upon the population density of *C. murinana,* and do not rise and fall in relation with *C. murinana* outbreaks. On the contrary, they were relatively much less numerous in the Vosges infestation than in the low population at Gernsbach. Pupal parasites may be of little value during a rise in the host population but play a considerable part in maintaining a population of *C. murinana* at a sub-economic level.

Three species have been reared at the Entomology Laboratory, Belleville, Ont., on a variety of lepidopterous pupae. These are *Itoplectis maculator*, *Apechthis resinator*, and *Pimpla turionellae*.

Itoplectis maculator (F.) gave rise to about half of all parasitism in the pupal stage in both areas of study. It is polyphagous, chiefly on tortricids and tineids. In the literature, it has generally been referred to the genus *Pimpla*.

Apechthis resinator (Thunb.) was second in importance in the Vosges, accounting for 25 per cent of pupal parasitism. *A. rufata* (Gmel.) was important only in the Vosges area.

Phaeogenes scutellaris Wesm. was important in both areas of study. The literature on other hosts of this species is scanty.

Pimpla turionellae (L.) occurred rarely in both study areas.

Monodontomerus aereus Wlkr. was the only chalcid parasite collected. It is polyphagous on lepidopterous larvae but may also act as a hyperparasite. Franz (3) found it to be the principal parasite in the Schwarzwald in 1939 but it was unimportant in the present study areas.

Nemorilla maculosa (Meig.) and *Bessa selecta* (Meig.) occurred rarely in the Vosges. Both are polyphagous and both have been previously introduced into Canada. *B. selecta* is best known as a sawfly parasite.

The following are first records for parasitism on *C. murinana: Apechthis resinator* (Thunb.), *Triclistus globulipes* (Desv.), *Pimpla turionellae* (L.), *Ichneumon pictus* Gmel., *Agrypon variitarsum* (Wesm.), *Metacoelus femoralis* (Geoffr.), *Chorinaeus tricarinatus* Holmgr., and *Pseudoperichaeta insidiosa* (R.D.).

THE VIRUS DISEASE

The most important biotic factor reducing the numbers of the European fir budworm in the field was a virus disease. Investigation on the etiology and epidemiology of this disease have been merely preliminary, and our present knowledge of the disease is far from complete. However, its importance in the field justifies a short review of the observations made.

The healthy, mature larva of *C. murinana* is about 2 cm. long, light green in colour, with a black head capsule and an orange anal shield. The skin is translucent so that the general pine-green body colour is a result of the green skin colour plus the light-green colour of the fat body and the blood serum.

Larvae displaying the typical symptoms of the virus disease were found in the field in the Vosges infestation as early as May 24, and appeared in the rearing trays at about the same time. The population was mainly in the sixth instar at this time and infected individuals were in this stage.

The first external symptom of the disease is a slight swelling of the body, without increased turgidity. There appears to be a small loss of muscle tonus so that each segment enlarges and the appearance of the segmentation is intensified; the insect is somewhat less responsive to stimuli. Then there occurs a gradual, progressive change in colour, which is the most distinctive symptom of the disease. The cuticle appears to become more transparent and to lose some of its green colour. At the same time, the fat body changes from green through yellow to white and

the blood from green to yellow. White particles appear in the blood, which becomes thickened and milky-yellow. The net result is that the over-all colour of the larva changes progressively from green to whitish-yellow. The change in colour is accompanied by reduced activity of the insect and increased flaccidity. The larva eats less and soon ceases to eat at all. Finally the insect dies. At death, it is very flaccid and the integument is fragile; the blood is thick and filled with small white particles, and most of the other organs are also full of these white particles. Shortly before death a milky-yellow fluid may exude from the anus or rarely from the mouth. This dries and sticks the insect to the substrate.

Frequently, insects die without undergoing the complete sequence of symptoms, yet microscopic examination demonstrates the presence of the typical white particles. Also, the typical symptoms are displayed only by sixth-instar larvae and by some fifth-instar ones. In the earlier instars the external symptoms are not clear. Dead third- and fourth-instar larvae show typical particles on microscopic examination without the typical colour change. Thus the external symptoms are only indicative of the presence of the disease and cannot be used exclusively as criteria of death from the virus.

The microscopic examination of the blood is a much more reliable method of demonstrating the presence of the virus disease. Symptoms appear here before they can be recognized with certainty externally. The first sign of abnormality is the presence in the cytoplasm of some blood cells of areas that appear to be filled with small, vibrating particles far beyond the range of resolution of the light microscope. These areas shimmer like hot air waves above a radiator. They can be seen only with dark-field illumination. Later, small highly refractive particles, undergoing intense Brownian movement, appear in the cytoplasm of the blood cells. These increase in number and aggregate until the whole cytoplasm is full. These particles are visible with ordinary light but are at the border of resolution and no clear picture of them can be visualized. They are indicated best by the dark-field, their high refractivity differentiating them from other particles in the cells. At this stage the symptomatic change in colour of the insect begins to become noticeable. The number of infected blood cells increases until all show the particles. The cells begin to rupture and the particles are liberated into the blood, which turns milky. On the death of the insect the blood is filled with these small, vibrating particles. At this time, cells of the fat body, hypodermis, and other tissues are also filled with particles and many cells have already disintegrated.

Long after the death of the insect, when the body has become hard, maceration of the tissues and examination with the dark-field reveals the presence of myriads of these highly refractive particles. They, therefore, form the best diagnostic feature of the disease. At this time, however, the picture may be complicated by the presence of other particles, so that unless the particles are present in large amounts it may be impossible to say with certainty that the virus has been the cause of death. Only a few of the dead larvae were examined microscopically at death; most examinations were made several weeks after the larvae had died. Only those insects in which the microscopic picture was unmistakable were considered to have

died of the virus. All questionable cases were considered as dying from unknown causes. Representative samples of dead *C. murinana* larvae were later examined under the electron microscope at the Laboratory of Insect Pathology, Sault Ste. Marie, Ont. These examinations confirmed the figures of virus mortality given in previous sections. Figure 3 is an electron micrograph of the particles in an unpurified maceration of a budworm larva that had been dead for nearly a year.

By microscopic examination, it is not possible at present to identify the presence of the virus disease in pupae. It is possible that the high mortality of pupae in the field is associated in some way with the virus disease, but this cannot be demonstrated by certain identification of the typical particles in dead pupae.

Bergold (2) prepared pure suspensions of the typical white particles of the virus disease, which he called "capsules". The electron microscope showed the capsules to be oval in shape, very dense, and of dimensions 360 × 230 mμ. When suspended in weak alkaline solutions (0.04M Na_2CO_3 plus 0.05M NaCL), the capsules dissolved and each liberated a single rod-like particle of dimensions 262 × 50 mμ. By analogy with the polyhedral virus disease of the silkworm, these rods are assumed to be the infectious agents of the disease, but no experimental proof of this assumption is available.

Preliminary infection experiments were undertaken to show that at least the intact capsules were infective. The incidence of virus infection and mortality in a sample of field-collected larvae was raised from 21 per cent to 72 per cent by feeding them suspensions of the capsules and to 89 per cent by inoculation of 0.005 cc. of weak capsule suspensions. In other laboratory experiments, performed on larvae collected as hibernating larvae in the field, feeding suspensions of the capsules resulted in 100 per cent mortality from the virus disease; control larvae, fed or injected with polyhedra of the virus disease of *Porthetria dispar* L., did not develop symptoms of disease.

Since this disease was discovered on European budworm larvae, similar virus diseases have been reported for other insects, among which are the variegated cutworm (6) and the buckeye caterpillar (7). Steinhaus (5) has created the genus *Bergoldia* to include insect viruses characterized by the presence of capsules or granules in the infected cells, and to the virus disease of *C. murinana* he has given the name *Bergoldia calypta*.

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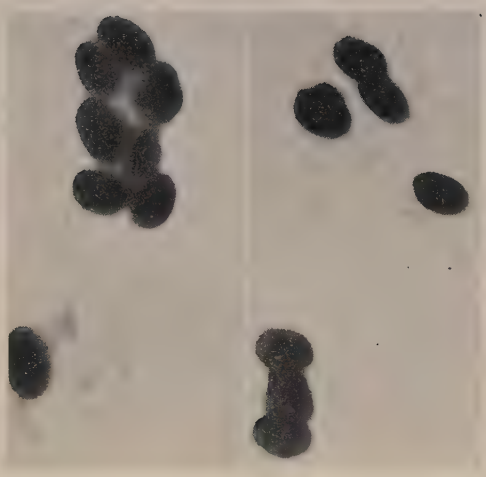

FIGURE 3. Virus capsules from *C. murinana* larvae. Electron micrograph of unpurified maceration from a larva dead for over a year (X20,000).

reviewed the German literature on distribution and J. Komárek, Department of Zoology, Karlovy University, Prague, Czechoslovakia, reviewed the Czech literature. E. Hubault, École Nationale des Eaux et Forêts, Nancy, made available the resources of the French Forest Service. Forstmeister Leonhard, Gernsbach, directed the German collections. G. Bergold, now at the Laboratory of Insect Pathology, Sault Ste. Marie, Ont., made available the facilities of his laboratory at the Kaiser Wilhelm Institut, Tübingen, Germany, and made many of the laboratory studies on the virus disease.

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METHODS OF BREEDING *AGROPYRON INTERMEDIUM*[1]

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ABSTRACT

Studies were conducted on relative seed-setting ability of 34 selected clones of *Agropyron intermedium* under open-pollination and when crossed and selfed under paper bags, in both field and greenhouse. In addition, the variation between characters of clones and their open-pollinated and polycross progenies was studied in replicated trials in the field. Association between characters, parent clones and progenies, and between various characters of the clonal lines, was found by means of correlation coefficients.

Clones differed considerably in ability to set seed, 17.7 per cent of the clones were practically female sterile. All clones were self-infertile, setting only an occasional seed. Cross-fertilization under bags was quite successful. A considerable quantity of aborted pollen occurred in a number of lines, but the association between aborted pollen and female infertility was very low.

The F test showed that there were highly significant differences between lines for all 20 characters studied in both clonal lines and progenies. Correlation coefficients indicated that the association between parent clones and progenies was much greater for morphological characters than for yield and vigour. Useful significant correlations were those between vigour and yield and plant density and yield.

INTRODUCTION

Intermediate wheatgrass *Agropyron intermedium* Host. (Beauv.) is rapidly becoming an important forage grass in the Prairie Provinces of Canada and in the Great Plains and Pacific Northwest regions of the United States. However, few improved strains are available and the bulk of the commercial production comes from strains of diverse origin.

Observation on spaced populations in introduction nurseries and in strain tests indicated to the writer that great variation in morphological characters occurs in this species. An examination of several old nurseries also indicated that certain plants had died much sooner than others due to winter injury, drought damage, or other causes. It was the realization of these wide differences between plants within the species, together with its growing importance as a forage crop and the possibility of developing improved strains for better adaptation to local conditions, that prompted the writer to undertake this study.

The primary objective of the problem was to study character variation between individual selected plants in replicated clonal nurseries and the extent of heritability of important character differences in replicated tests of their seed progenies. The interrelationships of various characters of selected clones were determined as well as the correlation of character expression in clones and in their seed progenies.

REVIEW OF LITERATURE

The literature reviewed is concerned, primarily, with *Agropyron intermedium* (Host.) Beauv., *Agropyron trichophorum* (Link) Richt, and other closely related Agropyrons. In addition, breeding studies on other forage crops which have a bearing on this study are briefly considered.

[1] Contribution from the Experimental Farms Service, Department of Agriculture, Ottawa, Canada. A condensation of a thesis submitted to the Graduate School of the University of Minnesota in partial fulfilment of the requirements of the degree of Doctor of Philosophy.

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Hafenrichter *et al.* (7) report that intermediate wheatgrass is adapted on soils with good drainage and of medium high fertility in the Pacific Northwest where rainfall is at least 15 inches. Wasser and Nelson (34) found that intermediate wheatgrass repeatedly outyielded crested wheatgrass and smooth bromegrass on clay loam soils under dryland conditions in Colorado. Franzke (6) stated that the species is ideally adapted to pasture and Adams *et al.* (1) recommend it, along with such grasses as crested wheatgrass and bromegrass, for general use on dryland in South Dakota.

According to Nevski (23), *Agropyron intermedium* is widely distributed in Middle Europe, the Balkans, and Asia Minor. It is adapted to high lime soils along slopes and on steppes. *Agropyron trichophorum* is native in the same general regions and adapted to the same soils. Both species are considered to be good forage plants, but poor seed producers.

Peto (24) points out that tremendous variability is recognized in most Agropyron species which have been studied. He also states that the taxonomy of the genus is in a very unsatisfactory condition, chiefly because authorities differ widely in their concepts within the genus. This he attributes to the wide range of variability existing within the so-called species. He found that the somatic chromosome number of *Agropyron glaucum* R. and S. was 42 and that of *A. pungens* (Pers.) R. and S. was also 42. The present writer considers these species to be synonyms of *Agropyron intermedium* (Host.) Beauv. Hartung (8) reported a strain of *A. intermedium* from Maikop, Caucasus, U.S.S.R. (F.P.I. 98568), as having 42 chromosomes while another strain from Bolu, Turkey (F.P.I. 109219), had 43 chromosomes. *Agropyron trichophorum* (F.P.I. 107330) had a somatic chromosome complement of 42. Myers (22) gives a complete review of the cytogenetics and genetics of forage grasses. In his Table 1, the chromosome number of *A. glaucum*, *A. pungens*, *A. trichophorum*, and *A. obtusiusculum* is given as 42.

In pollination and seed formation studies in grasses, Smith (27) found that *A. intermedium* set 0.007 selfed seed and 0.234 crossed seed per floret, while *A. trichophorum* reacted similarly, setting 0.012 selfed seed per floret and 0.227 crossed seed. Considerable variation was found between fruitfulness of different plants and inter-annual fluctuations were great when the same plants were studied in successive years. Fisher (5), working with *A. trichophorum*, found selfed seed set to be 2.2 per cent from selfing as compared with 12.7 per cent from hand cross pollination and 64.4 per cent from wind pollination. Jones and Newell (13) reported *A. intermedium* as having a pollen dispersal period of ten days per inflorescence, similar to that of crested wheatgrass and bromegrass. The optimum temperature for blooming and pollen dispersal appeared to be between 80° and 85° F.

In seed setting studies with bromegrass, Domingo (3) found that the most satisfactory cross-pollination was obtained by placing male and female inflorescences together under the same isolation bags or by transferring pollen collected on male inflorescences previously isolated in pollen bags. Knowles and Horner (16) investigated the effect of various methods of isolating inflorescences on the seed set in crested wheatgrass. They reported little seed setting under glassine and Kraft paper bags upon selfing, but when the heads from different plants were enclosed the seed set was quite good, indicating that low seed set on selfing was due to true self-sterility and not unfavourable conditions arising from bagging.

The principle of progeny testing, in one form or another, was first applied to grass breeding by Jenkin (10), Stapledon (29), and Kirk (14, 15). Jenkin (10) states that if plants are fairly highly self-fertile and there is little loss of vigour on selfing, the parent plants may be compared by these progenies. However, if this is not the case, it is necessary to intercross all the plants within a group in every possible direction and evaluate them on the basis of progenies resulting from the crosses. Stapledon (29), in his strain building, selected no less than 30 unrelated plants and tested 50 selfed progenies from each. The families breeding true to type were retained and the strain was built up from the best of these progenies. Kirk (15) considers that using cross-pollinated progenies from heterozygous parent plants to evaluate them offers great possibilities to plant breeders. He considers it to be of supreme importance to test under environmental conditions similar to those in which a variety presumably will be grown. Stevenson (30) describes methods of strain building of grasses and legumes involving the composite crossing of a number of parent plants which were carefully selected on the basis of progeny performance.

In recent breeding studies with cross-pollinated grasses, workers in general found important correlation coefficients between parent clones and clonal progenies and between clones and their cross-pollinated progenies. Associations between various characters were also established. Schultz (26) established that winter hardy plants of orchard grass produce winter hardy progenies. Murphy (19) found significant differences in yield, plant height, and root rot injury among plants of crested wheatgrass tested in replicated clonal progenies. He suggests that a replicated clonal trial may be a desirable means of selecting desirable clones. Hayes and Schmid (9) concluded that selection in selfed lines of *Bromus inermis*, *Festuca elatior*, and *Dactylis glomerata* practised for only a few generations seems a logical and efficient method of isolating relatively vigorous lines with homozygosity in important characters. Johnson and Miller (11) obtained highly significant differences between lines in percentage of total carotinoid pigments, beta carotene, and total chlorophyll in crested wheatgrass and bromegrass. Knowles (17), in studies on combining ability in bromegrass and crested wheatgrass, found correlation coefficients of $+.76$ or higher between yields of open-pollinated progenies and controlled crosses of crested wheatgrass, and states that the practice of selecting on the basis of open-pollinated progeny performance is justified for this grass. Further support for the reliability of the use of open-pollinated progenies in evaluating selected clones came from the significant correlations between open-pollinated progenies and those from controlled crosses regarding the creeping character in bromegrass and the aphis reaction character in crested wheatgrass. General combining ability was more important in crested wheatgrass, while specific combining ability was relatively more important in bromegrass.

Polycross nurseries for obtaining seed of a large number of plants for general combining ability tests were proposed by Tysdal *et al.* (32) in connection with alfalfa breeding. Bolton (2), studying combining ability of alfalfa, outlines a general procedure for selecting and testing for seed and forage yield. He states that seed from polycross nurseries is to be used in polycross tests to provide a rough measure of combining ability similar to

that provided by the top cross method of testing corn hybrids. Tysdal and Crandall (33) reported on the performance of polycross progenies of alfalfa in comparison to top crosses, average performance of single crosses and selfed progenies. The results point to the value of polycross tests for estimating general combining ability. Clones ranked very nearly the same for forage yield, bacterial wilt resistance, and leaf hopper resistance, no matter which progeny test was used to evaluate the clones. Knowles (17) found little advantage for polycross progenies over open-pollinated progenies in determining general combining ability of bromegrass clones.

MATERIALS AND METHODS

Plant Material

The materials used in this study consisted of 34 plants selected in 1947 from the original nursery of 210 plants, their clonal progenies, open-pollinated progenies, and polycross progenies.

The nursery had been established during 1946 with seed from two sources—South Dakota State College, Brookings, and Colorado Agricultural Experiment Station, Fort Collins. The seed from Brookings was of the Ree variety, which, according to Franzke (6), was brought to South Dakota by Wayne Austin under the name of *Agropyron pungens* P.I. 98568. It was planted in 1937 and the material of the strain collected in 1941 was classified by J. R. Swallen as a combination cross of *Agropyron intermedium* and *Agropyron trichophorum*. The seed from Fort Collins was of the Nebraska-50 strain which also was selected from the U.S.D.A. introduction P.I. 98568. This strain was developed by mass selection through three generations under isolated conditions.

The plants were selected for excellent vigour and leafiness, but showed considerable variation in many morphological characters. The intensity of selection was 10.7 per cent for the Ree variety and 17.8 per cent for the Nebraska-50 strain, indicating that the latter contained a greater number of superior plants under the prevailing conditions.

The selected plants were dug up early in May, 1948, and transferred to the greenhouse where they were sub-divided to produce the required number of plants for clonal progeny tests. The open-pollinated seed harvested from the plants in 1947 on the original site was used to seed the open-pollinated test and the polycross seed harvested in the greenhouse during 1948 was used for planting the polycross observation trial.

Establishment of Tests

The material was grown in the following tests, all of which were of the complete randomized block design:

(a) A clonal line test replicated twice was grown in the greenhouse during the summer of 1948. The plots consisted of single plants growing in gallon crocks. About one-third of the crown from each selected plant in the nursery was taken to the greenhouse and divided in half to give the two plants for the study.

(b) Another clonal line test replicated six times was grown in the greenhouse during the summer of 1950. The type of plots in this test was similar to those in (a). It has been the intention to establish this test in 1949 from the clones used in the first study. However, all plants failed to head out in 12 months even after various fertilizers were applied, the length of light hours increased, and the size of the clones reduced to eliminate the "sod-bound" condition, treatments reported to be effective in inducing heading in orchard grass by Sprague (28). Late in November 1949, two plants growing in crocks, of each of the 34 clones, were taken to a root

cellar where they remained in a dormant condition until April, 1950. The dormant period satisfactorily conditioned all plants so that normal growth was obtained and studies could be made on the material in 1950. Each plant from the root cellar was divided into three to give the six plants required for the greenhouse study.

(c) A clonal polycross nursery, replicated 16 times, was established in the field during 1949. A replicate consisted of the 34 clones in one row, randomized for position. The plants (plots) were spaced 3 feet apart in the row and the rows were 3 feet apart. Clones of equal size were started in 4-inch pots in the greenhouse on April 15 and transplanted to the field on May 27. Grasshoppers, principally *Camnula pellucida* (Scudd.), defoliated plants considerably throughout the season, but in October, when stand notes were taken, very few plants had been destroyed completely, although many were in a weak, unthrifty condition.

(d) An open-pollinated progeny test, replicated four times, was established in the field during 1948. Thirty-two progenies were included as insufficient seed from two lines necessitated their omission from the test. The Ree variety, the Nebraska-50 strain, and Fairway crested wheatgrass were used as checks. A plot consisted of a 12-plant row with the plants spaced 1 foot apart and the rows 3 feet apart. The seedlings were started in the greenhouse in paper bands 1 × 1 inch during March and transplanted to the field on May 3. A good stand was obtained.

(e) The polycross progeny test, replicated four times, was established in the field during 1949. The seed for this test was obtained in the greenhouse in 1948. Only 19 lines were included in this test because insufficient seed was harvested from the remaining ones. The Ree variety was included as a check. A plot consisted of one row of 12 plants spaced 3 feet apart. The rows also were spaced 3 feet apart. The seedlings were started in the greenhouse in paper bands 1 × 1 inch during February and transplanted to the field on May 9. A complete stand was obtained, but defoliation by grasshoppers throughout the summer and fall weakened plants considerably.

Studies of Seed Setting

The studies of seed setting were conducted in the greenhouse on clones in tests (a) and (b) and in the field on clones in the last eight replicates of test (c). These studies were made to determine the extent of selfing and crossing under bags of different plants as compared to seed setting under normal conditions.

Glassine bags were used to enclose heads to prevent cross-pollination and also to make crosses between different clones in the greenhouse. In selfing, a single head was enclosed in a bag $1\frac{1}{2} \times 7$ inches, while in crossing, four or more heads were enclosed in a bag 4 × 9 inches. The bags were securely tied at the base and to a stake to avoid breaking of the culm. They remained on the inflorescences until seed was harvested.

Parchment No. 5 paper bags were used in the field. These bags, $3\frac{1}{2}$ inches wide at the mouth by 12 inches long, proved to be long enough for the longest heads. In selfing, 10 heads of about the same maturity were enclosed in one bag, and in crossing, 10 heads were enclosed also, 5 from each of two plants. Special care was exercised in choosing heads of the same maturity for inclusion under one bag. Although 10 heads were enclosed in one bag, it was still necessary to tie the bagged heads to a stake to avoid breakage of culms or loss of bags through wind action. The bags were not removed until the seed was harvested.

A fan, driven by a 1/6 h.p. motor, was used to create air movement to effect pollination of unbagged heads in the greenhouse. The position of the fan was changed twice daily during the flowering period to ensure good distribution of pollen.

The seed set per head was determined by examining every head individually. The florets of heads containing very few seeds were pressed

between thumb and forefinger to determine whether or not the hull contained a caryopsis. This proved to be a very effective method. The heads setting a large number of seeds were threshed and then the seed was examined over a light (diaphanoscope) to separate the empty hulls from those containing a caryopsis.

A preliminary determination of relative seed set under open-pollination was made on ten heads of each plant selected from the original nursery in 1947. According to these results, the plants were classified into three groups, nearly sterile, medium fertile, and highly fertile. Five plants from each group were used in studies of crosses to determine inter and intra group fertility. The study was made in the test (b) greenhouse. In order to have certain plants adjacent to each other, it was necessary to shift the random arrangement of the clones slightly in three replicates of the test.

Relative pollen-sterility in 30 of the 34 clones was determined by the KI-I test by which normal and aborted pollen can be identified under the microscope. The aborted pollen, being devoid of starch, does not take on the stain, while normal starch-filled pollen turns very dark. The material for this study was collected in test (b) greenhouse. Two to four heads of each clone were taken just when the most mature anthers of a head were shedding their pollen. The heads were stored in a 70 per cent ethyl alcohol solution until the pollen studies were made several months later. Pollen counts were made on material from three anthers taken from three different spikelets. The pollen grains were counted and classified in four fields across each slide. The counts were made at the upper and lower edge of the drop and in two fields between. This seemed to give a representative count. There was only a slight tendency for aborted pollen to float to the edges of the drop. The average number of pollen grains studied per anther was 176. A magnification of 60× was used throughout the study.

Studies of Character Differences

The studies of character difference (except seed setting, pollen abortion, and pollen size) were from field trials of clonal lines in the first eight replicates of test (c), from open-pollinated progenies in test (d), and from polycross progenies in test (e). The pollen size was determined on the same material as was used in the pollen-sterility study.

In the study of clonal lines, each plot consisted of a single plant. In the study of the polycross progeny lines, each plot consisted of twelve plants and, although data were taken on individual plants, the plot means were used in the analysis. The characters of the open-pollinated progenies were studied on the plot basis, only one observation being recorded per plot.

The characters studied and the units of measurement or ratings were as follows: *hay yield*—pounds per acre; *seed yield*—pounds per acre; *seed weight*—grams per 100 hulled kernels; *dehulled seed*—per cent; *date of heading*—date when three-fourths of the heads had emerged; *plant height*—inches; *crude protein content* ($N \times 6.25$)—per cent; *winterkilling*—per cent; *winter injury*—per cent; *width of leaf*—the second leaf at the broadest point in millimeters; *plant density*—1 = densest, 5 = least dense; *fineness of stem*—1 = finest, 5 = coarsest; *foliage colour*—1 = blue, 2 = blue-green, 3 = green; *leaf pubescence*—1 = glabrous, 5 = most strongly pubescent; *head*

pubescence—1 = glabrous, 5 = most strongly pubescent; *creeping rootedness*—1 = bunch, 5 = most strongly creeping rooted; *stem solidness*—1 = hollow, 2 = intermediate, 3 = solid; *vigour*—1 = most vigorous, 5 = least vigorous; 6 = dead; *pollen size*—diameter in microns, thirty normally developed pollen grains were measured per line (ten per anther).

EXPERIMENTAL RESULTS

Seed Setting under Open-Pollination and Under Bags

A summary of data from greenhouse and field tests showing the average number of seeds per head obtained under open-pollination and under bags when cross- and self-pollinated is presented in Table 1. Although all but one of the tests in which seed setting studies were made were laid out according to a complete randomized block design and replicated, it was not possible to make a variance analysis of the results, at least not with all the lines, because of numerous missing plot values. Nevertheless, the scope of the study was sufficiently extensive to make the recorded data quite reliable and strongly indicative of what might be expected within the species in regard to seed setting. The data in the table are based on number of heads per line studies as follows:

Open-pollination:
1948 greenhouse 2–10 with 66 per cent of lines more than 5
1950 greenhouse 8–24 with 85 per cent of lines more than 16
1947 field 10
1950 field 10–35 with 75 per cent of lines more than 25

Under bags cross-pollination:
1950 greenhouse 2–20 with 85 per cent of lines more than 7
1950 field 5–30 with 80 per cent of lines more than 15

Under bags self-pollination:
1948 greenhouse 1–10 with 85 per cent of lines more than 5
1950 greenhouse 6
1950 field 10–70 with 75 per cent of lines more than 40.

The data show that under open-pollination 6 of the 34 clonal lines set little or no seed. The relatively few heads studied in the greenhouse in 1948 and in the field in 1947 gave a true picture of the seed setting ability of these lines. The seed set in the remaining lines was rather erratic from year to year as is indicated by the correlation coefficients at the foot of Table 1. Smith (27) and Myers (20, 21) found that seed setting in certain grasses frequently fails to show agreement from season to season.

The seed set under field conditions was better than in the greenhouse, possibly due to the longer heads rather than higher fertility under those conditions. The average number of seeds developed per head in the greenhouse was especially high in 1950, being 70 per cent that of the seed set under field conditions that year. The average seed set per head in the greenhouse during 1948 was rather low in comparison. This lower seed set may be partly due to the much smaller population in the test and consequent less uniform distribution of pollen.

To obtain an indication of the reliability of differences in seed set between clones, the data from 19 clonal lines (no data from the infertile clones were included) complete for all 6 replicates in the 1950 greenhouse

experiment were analysed statistically. The F test indicated that there were significant differences in seed set between lines. The top line set 34.5 seeds per head, while the low line set only 14.1 seeds per head. The least significant difference at the 5 per cent point was 9.9 in the test.

Considering the data from self-pollination under bags, it is evident that intermediate wheatgrass is quite self-sterile. In the greenhouse, where single heads were enclosed in a glassine bag to effect selfing, one or two seeds developed in several lines, the average for all lines being 0.02 seeds per head. In the field, where 10 heads were enclosed under one parchment

TABLE 1.—SEEDS PER HEAD UNDER OPEN-POLLINATION AND UNDER BAGS WHEN PLANTS CROSSED AND SELFED IN GREENHOUSE AND FIELD

Clonal Line No.	Open-Pollination		Controlled Cross-Pollination		Self-Pollination	
	Greenhouse Average 1948 and 1950	Field Average 1947 and 1950	Greenhouse 1950	Field 1950	Greenhouse Average 1948 and 1950	Field 1950
1	20.4	32.8	2.1	4.8	0.00	0.40
2	3.1	—	0.6	0.2	0.00	0.00
3	10.6	25.6	4.8	4.4	0.19	1.00
4	8.5	35.2	7.6	6.9	0.00	0.00
5	0.0	0.0	0.4	0.0	0.00	0.03
6	14.2	33.0	0.9	10.8	0.00	1.76
7	24.1	38.8	6.0	3.5	0.00	0.00
8	20.8	42.8	23.0	3.3	0.00	1.56
9	11.2	45.6	10.6	4.1	0.00	0.44
10	20.5	18.4	3.1	1.0	0.00	0.40
11	22.5	41.9	9.3	4.6	0.10	0.93
12	31.0	13.9	1.0	0.8	0.00	0.00
13	18.0	31.8	7.6	4.3	0.00	0.60
14	0.2	0.0	0.0	0.0	0.00	0.00
15	25.4	37.8	4.2	6.3	0.06	0.68
16	4.4	29.6	6.3	1.4	0.15	0.95
17	2.6	0.6	1.3	0.6	0.00	0.06
18	15.2	24.0	1.0	0.6	0.00	0.00
19	24.3	26.5	3.8	5.9	0.00	0.63
20	16.0	46.3	4.0	7.5	0.00	0.49
21	12.6	36.4	1.9	9.2	0.00	0.72
22	21.1	24.8	1.7	4.7	0.00	0.00
23	21.6	35.5	4.9	2.2	0.00	0.14
24	5.4	3.5	0.0	1.4	0.00	0.10
25	15.8	27.2	1.3	9.3	0.08	0.87
26	19.2	30.2	3.0	5.0	0.00	0.70
27	0.4	0.1	0.0	0.6	0.00	0.66
28	20.2	33.1	0.4	2.1	0.00	2.00
29	—	44.0	1.9	7.4	0.00	0.93
30	25.6	44.1	11.8	4.4	0.09	0.31
31	11.6	18.8	4.3	—	0.00	0.20
32	—	23.0	—	4.2	0.00	0.30
33	14.8	38.4	3.1	3.8	0.00	0.20
34	30.8	37.0	6.1	—	0.00	0.20
Mean	15.4	27.9	4.2	3.9	0.02	0.51

Corr. Coeff. 1948 and 1950 greenhouse seed set = +.527 sig. at 1% level.
" " 1947 and 1950 field seed set = +.197 not sig.
" " 1947 field and 1948 greenhouse seed set = −.129 not sig.
" " 1950 field and 1950 greenhouse seed set = +.351 not sig.

NOTE: 1. Data from clones 2, 5, 14, 17, 24, and 27 were not included in determining the above correlation coefficients.
2. 0.0 indicates less than 0.05.
0.00 indicates less than 0.005.

paper bag to effect selfing, the result was essentially similar, except that the majority of the lines produced a seed or two on one or more heads. The average seed set per head was actually 50 times greater in the field than in the greenhouse in 1950. It does not seem probable that contamination was responsible for this difference as holes in bags were noticed in only two instances. This relatively much higher selfed seed setting obtained in the field probably was due to the greater number of inflorescences enclosed under one bag. That better seed setting generally results when more than one inflorescence is enclosed in one bag has been reported by Frandsen (4).

When heads from different plants were enclosed under one bag, average seed set was the same in the greenhouse where glassine bags were used as in the field where parchment bags were used. This seed set was about 14 per cent of that set under open-pollination in the field, indicating the detrimental effect of the bag on seed setting and corroborating the results of others in this regard (16, 27). However, the number of selfed seed obtained under similar bags was only 13 per cent that of the cross-pollinated seed obtained, indicating that the plants of intermediate wheatgrass studied were truly self-infertile.

An examination of data presented in Table 1 shows that, in several cases, the seed set under bags was nearly as high as under open-pollination. For example, line 8 set 23.0 seeds per head under bags and 20.8 seeds per head under open-pollination in the greenhouse. Further, it was noted that on certain heads many seeds developed, while on others, from the same plant, none were formed. This suggests that the flowering period of the enclosed heads from one plant did not coincide with that of heads from the other plants.

Seed Setting in Special Crosses and Classification of Pollen Grains

Based on seed set under open-pollination in the 1947 field and 1948 greenhouse studies, fifteen clonal lines were classified into three classes according to degree of fertility, viz.:

I—*Nearly sterile*—lines 2, 5, 14, 17, and 27; seed set less than one seed per head.

II—*Moderately fertile*—lines 1, 3, 13, 25, and 31; seed set 8 to 14 seeds per head.

III—*Highly fertile*—lines 7, 11, 23, 30, and 34; seed set 25 + seeds per head.

The plants within a class were intercrossed and crosses were made between plants belonging to different classes.

In Table 2 the results are summarized according to inter and intra group crosses, with the pollen parent not identified any further than class. Three of the five nearly sterile plants produced no seed regardless of the pollen source, while the other two produced a few seeds. The moderately and highly fertile plants produced a similar number of seeds when fertilized with pollen from the other two classes.

In Table 3, the seed set is given for identified crosses. It will be noted that pollen from clone 2 (nearly sterile) and clone 17 (nearly sterile) failed to produce a single seed, while pollen from the other three female infertile clones (5, 14, and 27) did accomplish fertilization. In several cases, good

TABLE 2.—SEED SET OF CLONES FROM DIFFERENT FERTILITY GROUPS IN THE GREENHOUSE WHEN CROSSED WITH PLANTS IN THE SAME FERTILITY GROUP AND DIFFERENT FERTILITY GROUPS

Clone No.	No. Heads Studied	Av. No. Seeds Per Head	No. Heads Studied	Av. No. Seeds Per Head	No. Heads Studied	Av. No. Seeds Per Head
♀ Nearly sterile	X Nearly sterile		X Moderately fertile		X Fertile	
2	7	0	2	0	3	0
5	10	0	2	0	3	0
14	11	0	1	0	3	0
17	12	0.15	2	0	4	0
27	6	0	2	0	5	0.60
Average		0.03		0		.15
♀ Moderately fertile	X Nearly sterile		X Moderately fertile		X Fertile	
1	2	0	6	5.33	2	2.00
3	1	9.00	4	14.75	2	0.50
13	2	18.00	4	3.25	–	—
25	2	0	7	1.00	2	0
31	2	2.50	6	1.16	2	0.50
Average		5.90		5.06		0.75
♀ Fertile	X Nearly sterile		X Moderately fertile		X Fertile	
7	3	1.33	2	3.50	5	10.00
11	2	1.00	1	1.00	6	4.33
23	3	2.00	2	7.00	6	9.17
30	4	0	–	—	9	7.55
34	2	1.00	2	0	8	0.62
Average		1.07		2.88		6.33

Average seed set for 73 heads of plants of the nearly sterile group regardless of male = .07
Average seed set for 44 heads of plants of the moderately fertile group regardless of male = 3.95
Average seed set for 55 heads of plants of the fertile group regardless of male = 4.35

seed setting resulted. The data are not extensive enough to allow drawing definite conclusions, but it would seem that female sterility is not associated with male sterility in all cases.

Pollen classification, as to normality based on presence of starch, for thirty clonal lines is presented in Table 4. The results show that lines differed a great deal in percentage of normal pollen. The nearly sterile line, which failed as a pollen parent to produce a single seed, had 98 per cent aborted pollen grains which were completely hyaline and very small. However, the nearly sterile line 2, which also failed to effect fertilization, contained 52 per cent apparently normal pollen. Further, the nearly sterile line 14, which had no normal pollen but 43 per cent partly starched filled pollen, produced considerable seed as the male parent. Clone 5, which was completely female sterile, not having set a single seed under open-pollination in any test, had 78 per cent normal pollen, some of which was capable of fertilization. On the other hand, several highly fertile female clones, such as 22 and 29, contained a very high percentage of aborted pollen.

TABLE 3.—SEED SET IN IDENTIFIED CROSSES SUMMARIZED TO SHOW THE EFFECTIVENESS OF THE MALE TO FERTILIZE

Male—♂		Female—♀		No. Heads Studied	No. Seeds Per Head
Clone No.	Fertility Class	Clone No.	Fertility Class		
2	I	1	II	2	0
2	I	7	III	1	0
2	I	34	III	1	0
5	I	3	II	1	9.0
5	I	11	III	1	2.0
5	I	30	III	2	0
14	I	13	II	2	18.0
14	I	23	III	3	2.0
17	I	25	II	2	0
17	I	11	III	1	0
17	I	30	III	2	0
27	I	31	II	2	0.5
27	I	7	III	1	1.0
27	I	34	III	1	1.0
1	II	3	II	2	9.0
1	II	23	III	2	7.0
3	II	1	II	2	2.0
3	II	34	III	2	0
25	II	13	II	2	1.0
25	II	31	II	2	0
25	II	11	III	1	1.0
31	II	25	II	2	2.0
31	II	7	III	2	3.5
7	III	31	II	2	0.5
7	III	11	III	2	6.0
11	III	25	II	2	0
11	III	7	III	2	6.0
23	III	1	II	2	2.0
23	III	30	III	3	0.3
30	III	23	III	3	19.0
30	III	34	III	3	1.3
34	III	3	II	2	0.5

The correlation coefficient of +.377 between seed set under open-pollination (Table 1, field 1950) and per cent normal pollen (Table 4) was significant at the 5 per cent level. This further indicates that male and female infertility are not closely associated.

Character Differences between Clones, Open-Pollinated Progenies, and Polycross Progenies

Variance Analysis All Tests—The F test showed that there were highly significant differences between clonal lines for every character studied. The

FIGURE 1. A portion of Replicate 4, polycross test, showing differences in grasshopper damage to different lines. The line in Plot 83 (centre) was severely eaten down, while lines in Plots 82 and 84 were not eaten to any extent.

differences were apparent in the seed progenies also, but for several characters, such as fineness of stem and winter injury, significance was established only at the 5 per cent level.

Fall vigour notes were taken in October when the plants were still green. The differences in vigour at this time could be largely attributed to damage from roadside grasshoppers (*Camnula pellucida* Scudd.) which were quite abundant until mid-September. As it was difficult to rate for damage at intervals throughout the season because of insufficient damage at any one time to set up definite classes, it was thought to be more desirable to give a rating for vigour at the end of the growing season which would reflect a cumulative defoliation effect by the insects. General observations of the field trials throughout the season regarding grasshopper damage to the plants convinced the writer that defoliation by these insects, more than any other single factor, affected the vigour of the plants by the end of the first season. For this reason, the vigour data can be also considered indicative of relative grasshopper damage to the lines. Although continuous defoliation of plants was most severe around the edges of the tests bordering the roadways, the variance analysis showed that preferential feeding, as indicated by the vigour of the plants, was highly significant in both the clonal line and the polycrossprogeny tests (Figure 1). In placing any breeding significance on vigour, this has to be given serious consideration as the breeder, by selecting vigorous plants in the second year, could be overlooking valuable plants that were severely damaged in the first year.

TABLE 4.—CLASSIFICATION OF MATURE POLLEN GRAINS FOR 30 CLONAL LINES AS TO STARCH CONTENT

Clonal Line No.	Per Cent Pollen Grains			Clonal Line No.	Per Cent Pollen Grains		
	Normal Starch Filled	Semi-Normal Partly Starch Filled	Aborted No Starch		Normal Starch Filled	Semi-Normal Partly Starch Filled	Aborted No Starch
1	72	4	24	18	56	10	34
2	52	13	35	19	73	0	27
3	86	5	9	20	55	14	31
5	78	6	16	21	74	12	14
6	85	3	12	22	18	23	57
7	83	7	10	23	91	6	3
9	69	7	24	24	28	8	64
10	26	7	67	25	67	2	31
11	68	10	22	26	76	3	21
12	76	7	17	27	57	9	34
13	86	1	13	28	32	44	24
14	0	43	57	29	16	34	50
15	79	9	12	30	85	3	12
16	85	1	14	31	71	11	18
17	1	1	98	34	91	1	8
Average					61	10	29

TABLE 5.—FREQUENCY DISTRIBUTION FOR PLANT CHARACTERS OF 34 CLONAL LINES

The class centres are separated by 20 per cent of the difference between the two extreme means for the character

Character	Classes—Low to High					Range of Means	Mean All Lines
	I	II	III	IV	V		
Fall vigour	4	9	6	9	6	2.8 – 4.9	3.9 rating
Spring vigour	2	11	11	6	4	2.1 – 5.6	3.8 "
Foliage colour	6	5	12	1	10	1.0 – 3.0	2.1 "
Solidness of stem	2	4	3	3	22	1.0 – 3.0	2.6 "
Degree of creeping rootedness	4	11	7	8	4	1.5 – 5.0	3.0 "
Plant density	12	1	10	6	5	1.0 – 4.0	2.2 "
Fineness of stem	7	6	5	9	7	1.3 – 4.0	2.6 "
Leaf pubescence	14	6	5	4	5	1.0 – 5.0	2.5 "
Head pubescence	17	5	5	5	2	1.0 – 5.0	2.1 "
Hay yield	8	10	11	4	1	484 – 2606	1,299 lbs./acre
Height	1	5	7	8	13	28 – 44	37.7 inches
Date heading	3	10	9	3	9	June 25 – July 1	June 28
Degree of winterkilling	21	8	3	1	1	0 – 62	13.2%
Crude protein content	10	10	9	4	1	8.9 – 12.5	10.2%
Width of leaf	3	11	6	11	3	6.5 – 11.5	9.0 mm.
Seed weight per 100 kernels*	2	5	9	8	5	.48 – .86	0.69 gms.
Seed dehulling extent	6	6	13	2	2	5.7 – 57.4	26.8%
Pollen grain diameter	5	7	7	5	3	34.0 – 45.5	38.2 microns

* 29 lines only.

McBean and Platt (18), investigating differential damage to barley varieties by grasshoppers, established significant varietal differences in the amount of damage in 9 out of 12 tests.

FIGURE 2. Several different types of intermediate wheatgrass plants. *Upper left*—Clone 17; *upper right*—Clone 20; *lower left*—Clone 1; *lower right*—Clone 8. For density these plants rated 3, 5, 2, and 4, respectively. For fineness of stem, they rated 2, 4, 2, and 3. Note difference in height of lower two plants.

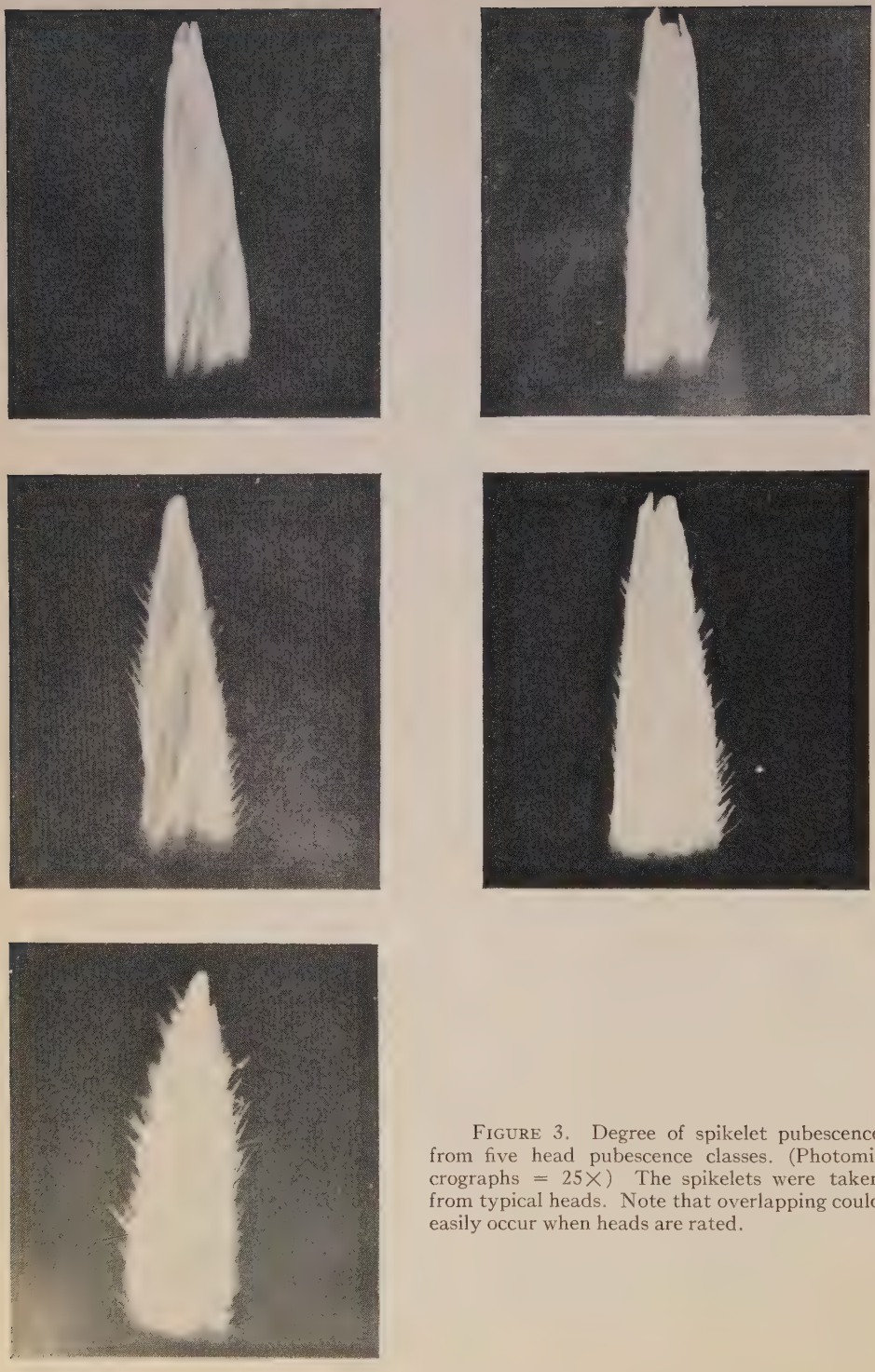

FIGURE 3. Degree of spikelet pubescence from five head pubescence classes. (Photomicrographs = 25×) The spikelets were taken from typical heads. Note that overlapping could easily occur when heads are rated.

Frequency Distribution for Plant Characters

Clonal lines—The data from the clonal lines have been arranged into five groups as shown in Table 5. Since very few characters were so distinct that the means of the lines all fell into a certain class, the distribution in the table has been made according to variation between the highest and lowest means for that character. Each frequency class comprises 20 per cent of the values between these extremes. For example, spring vigour means were from 2.1 to 5.6, a range of 3.5. This range divided by 5 gives the class interval of 0.7. Therefore, the classes set up for spring vigour are: I = 2.1 to 2.7; II = 2.8 to 3.4; III = 3.5 to 4.1; IV = 4.2 to 4.8; V = 4.9 to 5.5.

The frequency distribution shows that the means of the 34 selected clones for the various characters occur at different frequencies. For example, more lines were solid stemmed than hollow, and glabrous heads and leaves occurred more often than pubescent ones. The character having a distribution close to a normal one was seed and pollen grain size. The pollen grain diameters were generally smaller than reported for the species by Jones and Newell (13); this may be due to the different technique used in storing pollen or it may be due to environment, since they found that pollen size varied from year to year.

The variation in plant density and fineness of stem is illustrated in Figure 2 and that of head pubescence in Figure 3.

Open-pollinated progenies—The frequency distribution of the 32 open-pollinatproed genies in relation to the Ree variety and the Nebraska-50 strain is shown in Table 6. Class centres of the distribution are separated by multiples of the least significant difference at the 5 per cent level. The zero class contains all lines that did not differ significantly from the check strain for the character in question.

The data show that only three lines yielded significantly more than the Ree variety, while none yielded significantly more than the Nebraska-50 strain and twelve lines yielded significantly less than this strain. All lines but one were significantly outyielded by Fairway crested wheatgrass. This

TABLE 6.—FREQUENCY DISTRIBUTION FOR PLANT CHARACTERS OF 32 OPEN-POLLINATED PROGENIES

The class centres are separated by multiples of the Least Significant Difference (P = .05) arranged on each side of the 0 class which contains all progenies that did not differ significantly from the strain in question

Character	In Relation to Ree Class Centres					Range of Progeny Means	In Relation to Neb.-50 Class Centres				
	−2	−1	0	+1	+2		−2	−1	0	+1	+2
Hay yield (1949–50)			29	3		538 – 1490 lb./acre		12	20		
Winter injury (1948–49)			32			5.7 – 37.8% ($\sin^2 \theta$)			32		
Height (1950)		6	23	3		28 – 36 inches		10	22		
Crude protein (1950)		1	26	5		8.8 – 12.0%		2	26	4	
Colour of foliage			23	8	1	1.4 – 2.8 rating	3	14	15		
Plant density (1950)		2	26	4		1.3 – 3.3 rating			27	5	
Fineness of stem (1950)	3	7	22			1.2 – 3.2 rating		3	25	4	

NOTE: The 2-year average hay yield of Fairway crested wheatgrass in this test was 1715 pounds per acre.

may be an indication that intermediate wheatgrass is not as strongly competitive as crested wheatgrass and, when planted in widely spaced rows, cannot utilize the soil moisture between the rows as completely. It was quite apparent that more weeds grew close to intermediate wheat grass than to crested wheatgrass. The interaction between lines and years was significant.

Considerable winter injury was evident in the spring of 1949, which was preceded by a dry fall. Although the standard error of the mean for winter injury data was very great (44.9 per cent), chiefly due to the complicating factor of grasshopper damage being more severe around the edges of the test, significance was established between the extreme lines. However, not a single line was significantly more hardy than Ree or Nebraska-50. Crested wheatgrass suffered no winter injury in this test, indicating that intermediate wheatgrass is perhaps less hardy under dry conditions than crested wheatgrass.

Polycross progenies—The frequency distribution of nineteen polycross progenies in relation to the Ree variety for various characters is shown in Table 7. Here again, class centres of distribution are separated by multiples of the least significant difference. The class includes all lines not differing from Ree by the least significant difference at the 5 per cent level.

For certain characters, certain polycross progenies differed significantly from Ree. However, for such important characters as creeping rootedness, only one line was significantly less creeping rooted, and for yield, apparently no line was better than Ree. However, for seed yield, two lines were significantly better than Ree, one outyielded it by twice the least significant difference.

TABLE 7.—FREQUENCY DISTRIBUTION FOR PLANT CHARACTERS OF 19 POLYCROSSES

The class centres are separated by multiples of the Least Significant Difference, arranged on each side of the 0 class which contains all progenies that did not differ significantly from the Ree variety.

Characters	Class Centres					Range of Means	Mean All Lines
	−2	−1	0	+1	+2		
Vigour—fall		1	16	2		2.2 – 4.5	3.17 rating
Vigour—spring		4	10	5		3.2 – 5.5	4.05 "
Foliage colour		3	9	7		1.5 – 2.6	2.05 "
Degree of creeping rootedness		1	18			1.6 – 3.2	2.53 "
Plant density			19			2.8 – 3.8	3.34 "
Fineness of stem			17	4		3.1 – 4.0	3.43 "
Leaf pubescence			8	8	3	1.6 – 3.3	2.30 "
Head pubescence			12	5	2	1.4 – 3.8	2.51 "
Hay yield		2	17			1172 – 2166	1617 lb./acre
Seed yield*		1	13	1	1	60 – 173	84 lb./acre
Date of heading			19			June 30 – July 2	July 1
Winterkilling			16	3		8.9 – 46.1	19.1% ($\sin^2 \theta$)
Width of leaf			19			9.0 – 10.5	9.84 mm.
Weight per 100 kernels**		5	11	1		0.59 – 0.78	0.66 gm.
Seed dehulling extent**		1	16			11.1 – 30.1	20.7% ($\sin^2 \theta$)

* 16 lines.
** 17 lines.

TABLE 8.—CORRELATION COEFFICIENTS BETWEEN VARIOUS CHARACTER OF AGROPYRON INTERMEDIUM CLONES AND THE SAME CHARACTERS OF THEIR PROGENIES ALL GROWN IN THE FIELD

Character	Polycross Progenies		Open-Pollinated Progenies	
	n	r	n	r
Hay yield	19	.507*	32	.377*
Height			32	.428*
Protein content %			32	.305
Date of heading	19	.712**	32	.631**
Seed weight	17	.830**		
Dehulled seed %	17	.529*		
Fall vigour	19	.609**		
Spring vigour	19	.646**		
Winterkilling %	19	.452	32	.232
Colour of foliage	19	.891**	32	.776**
Density of foliage	19	.662**	32	.430*
Fineness of stem	19	.351	32	.473**
Width of leaf	19	.580**		
Pubescence of leaf	19	.603**		
Pubescence of head	19	.878**		
Creeping rootedness	19	.645**		

* Significant to the 5% point.
** Significant to the 1% point.

Correlation of Plant Characters in Clonal Lines with the Same Characters in the Progeny Lines All Grown in the Field

Indications of the heritability of the characters studied have been provided by correlation analyses between the means of clones with the means of their polycross and open-pollinated progenies. The results are presented in Table 8. In general the correlation coefficients between clones and polycross progenies were of a higher order than those between clones and open-pollinated progenies.

Based on parent-progeny relationships for yield, vigour, and plant density, it can be expected that yield may be improved by selecting high yielding, leafy, well tillered clones and combining them in a synthetic.

The correlation of clones with polycross progenies for fall and spring vigour was high and therefore, since fall vigour differences between lines were largely due to damage differences by grasshoppers, it can be concluded that whatever quality made the clones more palatable to grasshoppers was being transmitted to the progenies to a high degree through the female parent.

Exceptionally high correlation coefficients were obtained between parent clones and polycross progenies for colour of foliage and degree of head pubescence. It should be possible to concentrate these characters into a new strain relatively quickly. Such characters would be invaluable as markers in new strains.

To study the effect of clonal line vigour in relation to the yield of the polycross progenies, a partial correlation analysis was made using fall and spring vigour means from clonal lines and the yield of the polycross progenies The results are presented in Table 9. When the effect of fall vigour of clones was eliminated, spring vigour of the clones was still strongly associated

TABLE 9.—SIMPLE AND PARTIAL CORRELATION COEFFICIENTS OF FALL AND SPRING VIGOUR BETWEEN CLONE MEANS AND THE MEAN HAY YIELDS OF THE POLYCROSS PROGENIES

Characters	Simple Correlation	Partial Correlation
AB	.913**	.869**
AC	.564**	.062
BC	.595**	.496**

** Significant to the 1% point.
A = fall vigour of clonal lines.
B = spring vigour of clonal lines.
C = hay yield polycross progenies.

with the yield of the progenies, while when the effects of spring vigour of clones were eliminated there was no association between the yield of the progenies and the fall vigour of the clones. This indicates that fall vigour and spring vigour are really measures of the same thing, particularly when the high partial correlation between fall and spring vigour is considered in the interpretation.

Association of Plant Characters

Simple correlation coefficients were calculated from the data to determine the degree of association between various characters. If correlation coefficients between characters were significant for the clonal lines, they were calculated also for the polycross and open-pollinated progenies if data were available. All correlation coefficients were calculated from the means. The simple correlations are presented in Table 10, while partial correlation coefficients for several important characters of clones only are shown in Table 11.

Fall and spring vigour were closely associated, indicating that the condition of the plants in the fall has a lot to do with their condition in the spring. The simple correlations indicated a similar relationship of yield and fall vigour with such characters as plant density, fineness of stem, and protein content. The partial correlations, to a certain extent, clarify the complex situation of relationships between these characters. The plant density character had a significant association with yield even after the effect of all other characters was eliminated; similarly, fall vigour was associated with yield. The fineness of stem character was significantly associated with plant density when other effects were eliminated, but all its other associations proved insignificant. There was a significant negative association between hay yield and per cent protein content according to the partial correlation as well as the simple correlation, but the significant negative relationship of plant density and per cent protein content showing up as a simple correlation became a positive relationship, although not significant, when the effects of yield, fall vigour, and fineness of stem were eliminated. Vigour and plant density would seem to be the two agronomic characters that may be useful in selecting plants for breeding purposes.

In contrast to the findings of Tsiang (31) for bromegrass, height was not correlated with yield. This indicates that short plants are capable of yielding as much as tall plants. Width of leaf also had no bearing on yield,

TABLE 10.—CORRELATION COEFFICIENTS BETWEEN CHARACTERS OF CLONAL LINES AND OF CERTAIN OF THE SAME CHARACTERS IN THE PROGENY LINES

Comparison		Clones		Polycross Progenies		Open-Pollinated Progenies	
		No.of Lines	r	No.of Lines	r	No.of Lines	r
Yield of hay	and fall vigour	34	.824**				
" " "	and spring vigour	34	.900**	19	.758**		
" " "	and height	34	.007				
" " "	and plant density	34	.825**	19	.568*	32	.728**
" " "	and fineness of stem	34	.555**	19	.473*		
" " "	and width of leaf	34	−.056				
" " "	and degree of creep	34	.288	19	.369		
" " "	and % protein content	34	−.631**			32	−.266
Fall vigour	and spring vigour	34	.933**	19	.898**		
" "	and % plants winterkilled	34	−.709**	19	−644**		
" "	and plant density	34	.763**	19	.580**		
" "	and fineness of stem	34	.591**	19	.639**		
" "	and width of leaf	34	−.198				
" "	and degree leaf pubescence	34	−.000				
" "	and degree of stem hollowness	34	−.331				
" "	and % protein content	34	−.576**				
" "	and size of pollen	34	−.177				
Spring vigour	and degree of creep	34	.238				
" "	and degree leaf pubescence	34	−.063				
" "	and degree head pubescence	34	−.126				
% protein content	and blue to green colour	34	−.319				
" " "	and plant density	34	−.435**			32	−.082
" " "	and fineness of stem	34	−.381*			32	−.116
" " "	and width of leaf	34	.204				
" " "	and degree leaf pubescence	34	.051				
" " "	and date of heading	34	−.476**				
Height	and plant density	34	.175				
"	and fineness of stem	34	−.309				
"	and width of leaf	34	.426*				
"	and seed weight	29	.263				
"	and % dehulled seed	29	−.216				
"	and size of pollen	29	−.070				
Plant density	and fineness of stem	34	.734**			32	.795**
" "	and width of leaf	34	−.229				
" "	and seed weight	29	−.218				
" "	and % dehulled seed	29	.033				
Fineness of stem	and width of leaf	34	−.623**	19	−.142		
" " "	and seed weight	29	−.100				
" " "	and % dehulled seed	29	−.626**	18	−.352		
Width of leaf	and seed weight	29	.027				
" " "	and % dehulled seed	29	−.163				
Seed weight	and % dehulled seed	29	.009				
" "	and size of pollen	29	−.057				
Leaf pubescence	and degree head pubescence	34	.017				
Yield of hay	and yield of seed			17	.665**		
" " "	and winter injury					32	.008
('49 and '50)	('48 and '49)						

* Significant at the 5% point.
** Significant at the 1% point.

but it was significantly correlated with height, suggesting that the taller plants have the widest leaves. Tsiang (31) reported a similar relationship for height and leaf width in bromegrass.

An interesting relationship is the one of fineness of stem and per cent dehulled seed which gave a highly significant correlation coefficient of

TABLE 11.—SIMPLE AND PARTIAL CORRELATION COEFFICIENTS OF FIVE CHARACTERS OF CLONAL LINES OF INTERMEDIATE WHEATGRASS

	Simple Correlation		Partial Correlation
AB	.824**	AB.CDE	.539**
AC	.825**	AC.BDE	.598**
AD	.555**	AD.BCE	.255
AE	−.631**	AE.BCD	−.431*
BC	.763**	BC.ADE	.161
BD	.591**	BD.ACE	.115
BE	−.576**	BE.ACD	−.160
CD	.734**	CD.ABE	.574**
CE	−.435**	CE.ABD	.294
DE	−.381*	DE.ABC	−.180

* Significant at 5% point.
** Significant at 1% point.
Legend: A = hay yield.
B = fall vigour.
C = plant density.
D = fineness of stem.
E = % protein content.

−.623 for the clones and a non-significant correlation coefficient of −.352 for the polycross progenies.

There was no significant correlation between yield of hay of the open-pollinated progenies (1949 and 1950) and winter injury (1948–49). This suggests that differences in yield between lines were not strongly influenced by the winter injury suffered the first year after planting.

A number of morphological characters, such as leaf and head pubescence, colour of foliage, and solidness of stem, have very weak or no association with useful agronomic characters and, therefore, could not be used as selection guides. The colour of the foliage was studied specifically to find out if it could be used as a selection guide for selecting high protein plants, but in spite of a possible small association between high protein content and green foliage colour (in contrast to the bluish end of the colour variation), it would seem to be much too weak to be useful. Pickett (25), in a comprehensive study on association of morphological characters with protein content in bromegrass, came to the conclusion that none was a good enough indicator of high protein content to be useful in making selections.

Since a great many of the obvious morphological characters are not closely associated one way or another with undesirable agronomic characters, they could serve a useful purpose as markers on new strains that may be developed.

DISCUSSION

There was sufficient inter-plant variation in seed setting ability under open-pollination, in both field and greenhouse, to justify undertaking breeding work aimed at developing a better seed yielding strain of intermediate wheatgrass. The fact that 6 out of 34 clonal lines set practically no seed under the conditions of the tests points to the probability that

sterility factors may be rather widespread in the species. The high proportion of aborted pollen grains in a great many of the lines further suggests the possible existence of sterility factors and, in addition, possible irregular meiotic behaviour of the chromosomes. The fact that female fertility and per cent normal pollen only showed a slight association ($r = +.377$ significant at the 5 per cent level) indicates that low female fertility and male sterility do not necessarily go together. According to results from controlled crosses, certain of the female infertile clones produced pollen which was capable of fertilization and conversely a number of the female fertile clones produced highly sterile pollen. The results of this phase of the study point to the desirability of cytogenetic investigation in conjunction with breeding work undertaken with the species.

That a large portion of differences in seed set between lines is due to genetic variation is indicated by the seed yield data from the polycross progeny test. In this test, two lines yielded significantly more than the Ree check; one of these outyielded the check by more than twice the least significant difference.

No difficulty was experienced in obtaining good seed sets for all clones, except those classified as infertile, in the greenhouse when a fan was used to create air currents to distribute pollen. Preliminary experience, however, indicated a certain amount of difficulty in getting plants to head more than once in the greenhouse unless provided with a rather lengthy dormant period. Field results show that intermediate wheatgrass sets seed quite satisfactorily under the climatic conditions of the region.

A considerable number of seeds can be expected from controlled crosses under paper bags, either glassine or parchment, in both greenhouse and field. In this study, the average number of seeds set per head was 3.9 in the field and 4.2 in the greenhouse. This was 14.0 per cent and 27.3 per cent respectively of the amount of seed set under open-pollination. This compares favorably with that obtained for Fairway crested wheatgrass by Knowles and Horner (16). The very great variation in crossed seed setting under bags, sometimes for the same cross, indicates that the pollen dispersal period of heads from different plants often did not overlap sufficiently to ensure maximum fertilization. Knowles and Horner (16) attributed the relatively low seed setting of crested wheatgrass on crossing under bags to plants flowering at different dates. It should be possible to obtain better seed sets by being extremely careful in selecting the heads for similar stage of maturity when making crosses.

In the greenhouse, where single heads were isolated in bags, the number of selfed seeds obtained was negligible, while in the field, where ten heads were enclosed in a bag, seed set varied from a low of 0 seeds per head to a high of 2.0 seeds per head for clone 28. These results suggest that enclosing several inflorescences under one bag may give a greater number of selfed seeds than if a single inflorescence is isolated. Frandsen (4) found that this was true for a number of grasses. The low self fertility of intermediate wheatgrass as determined by this study generally substantiates the findings of Smith (27). The average selfed seed incidence was 1.5 per cent of that under open-pollination in the field as compared to 3 per cent reported by

Smith (27). The proportion of selfed to crossed seeds developed under bags in the field was 13.2 per cent. This is somewhat lower than that reported by Fisher (5) for stiff hair wheatgrass.

Because of the rather high degree of self sterility of this species and little variation between clones in this regard, emasculation should be unnecessary when making controlled crosses. It would be necessary to self a great number of heads to obtain an appreciable quantity of selfed seed. Nevertheless, it should be possible to obtain sufficient selfed seed of the most highly self fertile plants for limited progeny testing if at least 100 heads, in groups of ten, are bagged.

One of the main objectives of this study was to determine character differences between plants within the species *Agropyron intermedium*, and to determine to what extent the various characters were heritable. The clonal lines and their open-pollinated and polycross progenies were grown in three separate replicated field trials. The F test led to the conclusion that there were highly significant differences between clones and between their progenies.

The frequency distribution of the 34 clones for the range of the various characters shows that the distribution is rarely normal, suggesting that extreme types in the variation of a character occur more often in the strains from which the selections were made than those falling between the highest and the lowest value recorded. The frequency distribution of the open-pollinated progenies in relation to the Ree variety and the Nebraska-50 strain shows that Nebraska-50 is possibly the better of the two as no lines significantly outyielded it, while three significantly outyielded the Ree variety. This, in part substantiates the soundness of the basis for selection used in the original nursery when Nebraska-50 provided a greater percentage of what were considered vigorous desirable forage type plants than the Ree variety. In the polycross progeny test, not a single line significantly outyielded the Ree check. It is considered that the yield data were rather unreliable as a basis for selection because of the complicating influence of fall vigour on yield. Fall vigour differences between means of lines were considered to be largely due to preferential feeding by grasshoppers (*Camnula pellucida* Scudd.) and, consequently, lower yields resulted for those lines eaten down most severely. A considerable number of the progenies differed significantly from the check strains in many of the other characters studied. The greatest variation was evident for certain morphological characters, notably foliage colour, pubescence of head and leaf, and seed size.

The correlation studies between parent clones and progenies show that most characters are heritable to a considerable extent. Hay yield, seed yield, and winter killing were heritable to a lesser extent than vigour and most morphological characters. The fineness of stem character was not demonstrated to be due to genetic variation according to the analysis. The correlation coefficients between clones and progenies for yield were significant and of a somewhat higher order than those obtained by Knowles (17) for crested wheatgrass and bromegrass, and by Tsiang (31) for bromegrass. The correlation coefficient for the creeping rooted character between clones and polycross progenies in intermediate wheat grass was +.645

significant at the 1 per cent level as compared to +.74 for bromegrass reported by Knowles (17). This indicates approximately similar heritability for the creeping character in the two species.

The correlations between means of clones and their open-pollinated progenies were slightly lower, for five out of six characters, than between means of clones and their polycross progenies. This indicates that the polycross progenies were the better of the two for determining general combining ability of the clones. However, keeping in mind that the open pollinated progenies were planted one year earlier and hence relatively older plants were being compared with the clonal lines, they can be regarded as also giving a good indication of the breeding behaviour of the parent clones. Knowles (17), who used open-pollinated progenies, polycross progenies, and controlled cross progenies to evaluate selected clones of bromegrass and Fairway and Standard crested wheatgrass, came to the general conclusion that evaluating selections on the basis of open-pollinated progeny performance was entirely justified.

Since the variation between plants in *Agropyron intermedium* is great, it would seem to be desirable to plant clones of similar type in polycrosses and then compare their progenies in polycross tests. Combining clones in synthetics on the basis of polycross progeny performance tests offers a promising method for developing improved strains. A replicated polycross nursery might also be used to advantage in separating out into several polycrosses different types of plants. As there is considerable evidence of winter killing and drought injury in the species, it would be essential to study the progenies to an age of three years or more in order to find the lines damaged the least by drought and winter injury.

In view of the great diversity of type and relatively good heritability of important characters, mass selection for a desired type through several generations also offers possibilities worthy of consideration.

The association of plant characters was studied by calculating correlation coefficients on means of clones and also on their progenies if an association proved significant for the clones. Plant density was the morphological character closely associated with yield and vigour. Since it was based on leafiness and degree of tillering, selecting for leafy, strongly tillering plants should result in desirable progenies. Winterkilling is closely associated with fall vigour and cognizance should be taken of the reason for differences in fall vigour between plants. As it happened during this study, grasshoppers damaged certain lines more than others and, consequently, many of the lines most palatable to the grasshoppers suffered most winter injury. Actually, very desirable lines may be discarded if selection is practised during exceptional environmental conditions.

Protein content was significantly negatively associated with yield. There was no morphological character that could be used as indicative of high protein content. Pickett (25), in studies with bromegrass, also concluded that morphological characters could not be used as a selection guide for protein content.

There are a number of characters such as leaf and head pubescence and colour of the foliage which could serve a useful purpose as markers in any new strain that may be developed. None of these characters was closely

associated in one way or another with undesirable agronomic characters. All three characters showed a strong correlation between parent clones and progenies and it should not be difficult to fix them in a strain. A marker character on a new strain would be particularly useful in this new grass in order to maintain its identity without too much difficulty.

In a relatively new cultivated grass such as intermediate wheatgrass, it remains to be determined which end of the variation of certain characters is most desirable. For example, strongly creeping rooted strains may be desirable in one climatic region but not in another or in a pasture type but not in a hay type. Similarly, large seeded strains which have the ability to emerge from greater depths may be most useful in arid climates where establishment is difficult, while smaller seeded types may be satisfactory in more humid regions.

The possibilities for improvement in intermediate wheatgrass appear to be great and offer a challenge to plant breeders wherever the grass is adapted.

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SOIL POROSITY AND CORN YIELDS UNDER NINE THREE-YEAR ROTATIONS[1]

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ABSTRACT

The effect of nine different 3-year rotations on the distribution of soil pores and on yield of corn was studied. The rotations exerted a significant effect on the non-capillary pores but failed to affect the total soil porosity, indicating that the non-capillary pores increased at the expense of capillary pores. The percentage of large pores under the cropping systems where all crop residues were returned to the soil was not different than where the residues were removed and ten tons of manure added in the rotation. Where soybeans were grown before corn in the rotation the volume of large pores in the soil under corn was reduced. There was no definite relationship between the porosity of the soil and corn yields.

INTRODUCTION

The ability of a soil to produce is dependent upon many factors among which climate, soil fertility, physical condition and management are basic.

Maintenance of the productivity of the Brookston clay for corn has become more and more difficult. Corn yields in Essex County are reported (8) to be approximately 37 per cent lower for the 1945–50 period than for the period 1920–25. It is assumed that the climate of the county has not changed materially during the period in question. The use of chemical fertilizers has been on the increase during the same period so that lower yields have not been due to declining fertility alone. It has been suggested that productivity has declined as a result of the unfavourable effect on the physical properties of the soil through an increase in the production of row crops.

The purpose of this study was to measure the effects of nine different 3-year rotations, with and without fertilizers, on the porosity of the soil and yield of corn. It was believed that such a study might indicate, to some extent, the components of a rotation necessary to improve the physical condition and the productivity of the Brookston clay.

It has been shown (2) that the non-capillary porosity of the Brookston clay under continuous cropping to bluegrass was greater than where corn was grown continuously. Non-capillary porosity under second-year hay following corn and small grains was intermediate between continuous corn and continuous bluegrass. Page and Willard (9), working in Ohio, reported that continued cropping to small grains including soybeans resulted in declines in production as well as in favourable soil structure. Although aggregation and porosity were satisfactorily correlated with yield, neither measurement characterized soil structure well enough to explain all observed differences in growth. Cotton yields have been shown to vary with soil porosity although the correlation did not quite reach statistical significance (6). Baver (1) showed that poor physical condition in a soil affected the efficient use of fertilizer by restricting root development, impairing normal absorption by established roots and by impeding microbial activity.

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It has been shown that legumes grown continuously or in rotation (4, 7, 11) tend to conserve soil nitrogen as compared to the production of non-leguminous row crops.

MATERIALS AND METHODS

Nine different 3-year rotations were established on the Brookston clay at the Dominion Experimental Substation, Woodslee, in 1947. The Brookston clay exhibits the properties of the Dark Grey Gleizolic Soils (10). It has poor natural drainage and a fairly high organic matter content at the surface under natural conditions. The parent material has been classed as a heavy ground moraine which has been altered to a greater or lesser extent by wave action and lacustrine deposition. The rotation plots were established in duplicate with each plot subdivided so that one-half received 300 pounds of a 2–12–10 fertilizer per acre on all crops except alfalfa. The remainder of each plot has received no fertilizer since the rotations were established. In the spring of 1951 one-half of both the fertilized and non-fertilized portions of the plots were limed at the rate of 4000 pounds per acre. The lime and fertilizer treatments were applied in strips to facilitate cultural operations. For this reason the sub-treatments were not randomized.

Each crop of each rotation appeared every year. The plots sampled for this study were from the range growing corn in 1951. In this way all plots sampled were growing the same crop thus eliminating the effect of the current crop. All plots were fall ploughed prior to planting corn so that cultural treatments were uniform during the 10-month period preceding sampling. The rotations studied are as follows:

1. Corn-oats (alfalfa+brome)** -alfalfa+brome.
2. Corn-oats (sweet clover) -sweet clover.
3. Corn-soybeans-oats (sweet clover).
4. Corn-corn-oats (sweet clover).
5. Corn-oats (sweet clover) -soybeans.
6. Corn-oats (alfalfa) -alfalfa.
7. Corn-soybeans-soybeans.
8. Corn-oats (alfalfa+brome) -alfalfa+brome.*
9. Corn-oats (sweet clover) -sweet clover.*

In rotations 3 and 4 the oat straw was ploughed into the soil along with the seedling sweet clover so that only the grain was removed the year preceding corn.

Porosity samples were taken, using a hammer-driven core sampler with brass cores three inches in diameter and three inches in height. The samples were taken during the month of August and at a depth to represent the three- to six-inch layer of soil. One sample was taken from each of the four treated areas within the rotation plot. The brass cores were taken to the laboratory and saturated with water immediately by allowing to stand in water to a depth of 2.75 inches overnight. The following morning the cores were removed from the water and weighed. The cores were then placed on a tension table similar to that described by Leamer and Shaw (5) for a period of 24 hours. The volume of water drained from the sample by a tension of 40 centimetres of water (pF 1.60) was considered to be a

* All crop residues removed and 10 tons of manure added prior to corn.
** Crops shown in brackets indicate seedling stage.

measure of the volume of non-capillary pores. The total volume of water lost between saturation and oven dryness was taken as a measure of total pore space. The difference between total and non-capillary porosity is a measure of capillary porosity.

EXPERIMENTAL RESULTS

The Effect of Rotations on Soil Porosity

Table 1 shows the distribution of total soil porosity into capillary and non-capillary porosities for each of the nine rotations.

TABLE 1.—SOIL POROSITY (AS A PERCENTAGE OF THE TOTAL SOIL VOLUME) UNDER CORN GROWN IN NINE DIFFERENT THREE-YEAR ROTATIONS

Rotation	Non-capillary Porosity, %	Capillary Porosity, %	Total Porosity, %
1. C-O (A+B)–A+B	10.6	34.6	45.2
2. C-O (S.C.)–S.C.	10.1	34.2	44.3
3. C-Soy-O (S.C.)	11.9	30.0	41.9
4. C-C-O (S.C.)	12.8	31.9	44.8
5. C-O (S.C.) -Soy	8.0	37.2	45.2
6. C-O(A)-A	13.6	30.4	44.0
7. C-Soy-Soy	9.2	34.9	44.1
8. C-O (A+B)-A+B*	10.8	35.0	45.7
9. C-O (S.C.) -S.C.*	9.6	35.6	45.2
L.S.D. at P.05	2.5	2.8	N.S.D.

* All crop residues removed and 10 tons of manure added prior to corn.

These results indicate that the rotations have significantly affected non-capillary and capillary porosity, but not total porosity of the soil. Capillary porosity has been lowered by the same rotations that increased non-capillary porosity. There was a significant negative correlation between capillary and non-capillary pores ($r = -0.93$).

The soil under corn grown in rotation with oats and alfalfa had a significantly greater non-capillary porosity than that under any other rotation except the rotation corn-soybeans-oats plus sweet clover and the rotation corn-corn-oats plus sweet clover. The addition of brome grass to the alfalfa in the rotation corn-oats-alfalfa reduced the non-capillary porosity.

The rotation corn-oats-alfalfa plus brome grass and the rotation corn-oats-sweet clover resulted in the same porosities where the crop residues were removed and manure added as where all residues were returned to the soil. Rotations No. 3 and No. 5 included the same crops but the sequence of crops varied. In rotation 3 where oats and sweet clover preceded the corn the non-capillary porosity of the soil under corn was greater than where the soybeans preceded the corn crop as in rotation five. Comparison of these two rotations also indicates that the effect of sweet clover grown to the seedling stage had disappeared by the second year. The volume of large pores was low in rotation 7 where corn was grown in rotation with two years of soybeans.

The Effect of Commercial Fertilizer and Lime on Soil Porosity

The average soil porosity for each soil treatment applied to all rotation plots is shown in Table 2.

TABLE 2.—AVERAGE SOIL POROSITY UNDER LIME AND FERTILIZER TREATMENTS

Soil Treatment	Non-capillary Porosity, %	Capillary Porosity, %	Total Porosity, %
None	9.8	34.4	44.2
4000 lb. lime	9.5	34.7	44.2
300 lb. 2-12-10	11.8	33.3	45.0
4000 lb. lime plus 300 lb. 2-12-10	11.9	33.8	45.7
L.S.D. at P .05	2.0	N.S.D.	N.S.D.

Application of two tons of limestone per acre failed to cause any significant change in the volume of large or small pores in the soil. Application of 300 pounds of 2–12–10 per care increased the volume of non-capillary pores from 9.8 to 11.8 per cent. This increase just reaches statistical significance. Where both lime and fertilizer were applied the increase in non-capillary porosity was also significant. None of the soil treatments had any significant effect upon capillary or total porosity although there was a trend toward a higher total porosity and lower capillary porosity where fertilizer was used.

The Effect of Rotations, Commercial Fertilizers and Lime on the Yield of Corn

The yields of corn obtained from the rotation plots in 1951 are recorded in Table 3 as bushels of shelled corn per acre on the basis of 15 per cent moisture in the grain.

TABLE 3.—YIELD OF SHELLED CORN IN BUSHELS PER ACRE (1951)

Rotation	Soil Treatment				
	Check	4000 lb. Lime	300 lb. Fertilizer	4000 lb. Lime plus 300 lb. Fertilizer	Rotation Mean
	Bu.	Bu.	Bu.	Bu.	Bu.
1. C-O-A+B	31.9	38.8	47.8	50.1	42.2
2. C-O-S.C.	29.3	36.8	44.6	47.5	39.6
3. C-Soy-O+S.C.	28.9	35.2	32.7	37.8	33.7
4. C-C-O+S.C.	29.2	29.4	33.4	35.3	31.9
5. C-O+S.C.-Soy	31.6	42.7	40.3	46.5	40.0
6. C-O(A)-A	42.9	43.8	52.5	56.0	48.8
7. C-Soy-Soy	37.7	47.2	37.6	47.4	42.5
8. C-O-A+B*	42.6	49.9	38.2	51.6	42.6
9. C-O-S.C.*	38.8	38.0	44.8	52.4	43.5
Treatment Mean	34.8	40.2	41.3	47.2	
L.S.D. at P .05	For Rotation Means 6.50			For Treatment Means 5.70	

* All crop residues removed and 10 tons manure added prior to corn.

The rotation corn-oats-alfalfa produced 48.8 bushels of shelled corn in 1951 which was the highest yield recorded. This yield significantly exceeded that obtained in rotations 1 to 5 inclusive and exceeds the yield of rotations 7, 8, and 9 by an amount approaching the necessary difference for significance. The rotation corn-soybeans-oats plus sweet clover yielded significantly less corn than all rotations except No. 2, 4, and 5. The rotation corn-corn-oats plus sweet clover produced less corn than any of the other rotations except number three.

The portions of the plots which received lime or fertilizer yielded more corn than the check areas. Where lime and fertilizer were used in combination the yield was higher than where either was used alone.

DISCUSSION AND CONCLUSIONS

The non-capillary porosity was the same under the following two methods of management: (1) all crop residues removed and ten tons of manure added prior to planting corn and (2) all crop residues were returned, and no manure added. It would appear that the practice of returning all crop residues in the rotation was just as effective in maintaining non-capillary porosity as applying 10 tons of manure. Where lime and fertilizer were used the application of ten tons of manure failed to increase yield. However, on the check area where no lime or fertilizer was used an application of manure increased corn yields about 10 bushels per acre. These facts would indicate that manure is not essential in a three-year rotation providing crop residues are returned to the soil and commercial fertilizers are used.

The position of a soybean crop in the rotation is important in so far as maintenance of non-capillary porosity for the corn crop is concerned. When soybeans precede the corn crop the volume of large pores in the soil under the corn crop is reduced. If, however, the soybeans follow the corn, and oats plus sweet clover are grown prior to the corn crop, the volume of non-capillary pores in the soil under corn will be much greater. In spite of these variations in the soil porosity the yield of corn was significantly higher where the soybeans preceded corn. This may be due to some conservation of existing soil nitrogen by the soybean. The rotation corn-soybeans-soybeans resulted in low soil porosity but maintained the yield of corn at a level not significantly lower than the best yield obtained. Here again the relatively good yields of corn, in spite of low non-capillary porosity, may be due to soil nitrogen conservation.

The largest volume of non-capillary pores and the greatest corn yield was obtained from the rotation corn-oats-alfalfa. In a general way it may be said that all rotations in which a legume grown to maturity preceded the corn crop the best corn yields were obtained. All legumes, except soybeans, left the soil with a relatively large volume of non-capillary pores. The small volume of large pores following soybeans is probably a result of the smaller root system. Rotations 3 and 4 were effective in improving the non-capillary porosity but failed to give good yields of corn the following year. Seedling sweet clover in the oats, to be ploughed under as a green manure, may have helped to improve the non-capillary porosity. The low yields of corn may be a result of inadequate nitrogen supplies. The wide carbon to nitrogen ratio of the oat straw turned under would tend to tie up some of the soil nitrates.

The average volume of non-capillary pores on all areas receiving no soil treatment was not significantly different from the areas receiving two tons of calcitic limestone. The fertilized portions of the plots contained more non-capillary pores than the non-fertilized areas. This is probably due to the increased growth of the plants (3). The increased top growth would return more organic residues to the soil and the larger root systems would have an increased granulating effect on the soil. Corn yields were highest on the areas receiving lime and fertilizer and lowest on the check areas while the individual lime (only) and fertilizer (only) treatments were intermediate between the two.

There was a significant negative correlation ($r = -0.93$) between the capillary and non-capillary pores indicating that non-capillary porosity increases or decreases at the expense or benefit of the capillary pores. There were no significant correlations between soil porosity and corn yields. This may be more logical than it would appear at first since the same factors which are influencing soil structure may also be affecting the level of one or more nutrients in the soil. If this were the case some of the variations in yield would be due to changing nutrient levels as well as changing porosity. Under such conditions it would not be possible to obtain high correlations between soil porosity and yield. It would be interesting to know how these rotations would affect the physical properties of the soil if the nutrient variable were removed.

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STUDIES ON BLACK STEM OF ALFALFA CAUSED BY *ASCOCHYTA IMPFRFECTA* PECK[1]

I. SEED AND SEEDLING PHASES OF THE DISEASE

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ABSTRACT

Ascochyta imperfecta was shown to be seed-borne on over 75 per cent of the seed samples received from the alfalfa seed-growing areas of northern Saskatchewan. The fungus was found to be present in the form of mycelium on the outer surface of the seed coat. In experiments with artificial inoculation of seed with spores of *A. imperfecta* it was shown that mortality of seedlings as high as 90 per cent could occur under moist conditions in *in vitro* tests. Treatment of infected seed with Arasan, Semesan, Ceresan and Spergon reduced the average percentage of isolates from alfalfa seed from 23 to less than 1. Tissues of the hypocotyls and cotyledons of diseased seedlings were studied by means of microtome sections. Examination of these sections showed that mass invasion of the cortical tissues of the hypocotyl had occurred and that the tissues had disintegrated. The fungus massed in walled, pycnidium-like structures on the dead tissues. The mesophyll of the cotyledons was ramified with mycelium of *A. imperfecta*. Infection of this area killed the growing point and prevented further growth of the infected seedlings.

Black stem of alfalfa has been described and the associated fungi have been listed by various investigators (1, 2, 3, 4, 5, 6, 7). The most recent detailed study by Cormack (2) in Alberta indicates that *Ascochyta imperfecta* is primarily a parasite of the leaves and stems of alfalfa and is of doubtful importance on other naturally infected hosts. This fungus was found to be soil- and seed-borne and also capable of persisting on dry stems and leaves of alfalfa for at least five years.

Surveys of the alfalfa-growing areas in northern Saskatchewan have shown that the disease is widespread there and has increased in severity during the last five years. An important factor in this increase was the favourable nature of two cool moist growing seasons in succession, namely 1950 and 1951. Such seasons favour the development of the inoculum on stubble, fallen leaves, straw and the current seasonal growth and also make it difficult to burn infected trash in the spring. The examination of seed samples from many localities in the alfalfa seed-producing areas of Saskatchewan has supplemented field observations. The results of this examination show that all of the seed samples were infected with *A. imperfecta* and that infection tends to increase from slight in young fields to severe in old fields.

This paper presents the results of studies of black stem of alfalfa from the standpoints of seed infection, seed treatment, and seedling inoculation in the greenhouse and laboratory.

MATERIALS AND METHODS

In a laboratory study of *A. imperfecta* as a seed-borne pathogen of alfalfa, the seed was plated without surface sterilization on acidified potato

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dextrose agar in Petri dishes and incubated at 24° C. for 10 days. At the end of this period, the olive green colonies of *A. imperfecta* could be identified readily.

The form in which alfalfa seed carries *A. imperfecta* was investigated by washing the seed with water and plating drops of the water on potato dextrose agar, and by microscopic examination of portions of alfalfa seed coats which had been cleared in pyridine.

In the seed treatment experiments, the fungicides were applied to the seed in the form of dusts before the seed was plated on agar, and the relative efficiency of the fungicides was measured by the suppression of fungus growth from the treated seed as compared with that from the untreated seed.

The material for the seedling inoculation experiments consisted of alfalfa seed disinfected with calcium hypochlorite solution, washed, and inoculated with a spore suspension of *A. imperfecta*. Such seed was grown on moist filter paper, or in moist sand or soil. Data on infection and blighting of the seedling were obtained after 10 days. The manner of infection of the seedling was studied by means of stained microtome sections of the hypocotyl, cotyledons, and growing point.

EXPERIMENTAL RESULTS

Isolation of A. imperfecta from Alfalfa Seed

Samples of seed of the 1946 crop from 33 locations and of the 1947 crop from 18 locations in northern Saskatchewan were plated on agar. The occurrence of *A. imperfecta* was recorded and is summarized in Table 1.

TABLE 1.—PREVALENCE OF *A. imperfecta* IN SEED SAMPLES OF ALFALFA GROWN IN NORTHERN SASKATCHEWAN

Crop	Number of locations	Number of samples	Number of samples infected	Percentage seeds infected	
				Range	Average
1946	33	40	25	0–33	12.0
1947	18	18	18	3–30	10.9

The data in Table 1 indicate how widespread the disease is in northern Saskatchewan and how commonly *A. imperfecta* occurs on alfalfa seed. A comparison of the percentage of seeds infected in the 1946 and 1947 samples from these fields indicated that, in 1947, infection of the seed increased at 10, decreased at 4, and remained unchanged at 4 locations. The incidence of infection was observed to be related to age of stand. Several of the samples were taken from relatively young fields in both years and it was in these fields that the lightest infection occurred, while seed from old fields was infected more severely.

The Form of Inoculum of A. imperfecta on the Seed

Growth of *A. imperfecta* from unsterilized alfalfa seed on agar was found, in the experiments described in the previous section, to be fairly rapid. Hyphae grew out into the agar from the seed in 12 to 18 hours and

examination of these hyphae showed that they arose from portions of the seed coat which were in contact with the agar. These hyphae could develop from spores on the surface of the seed coat or from mycelium on or in the seed coat. The form of inoculum on the seed was investigated by washing 2-gram lots of infected samples in sterile water, examining the washings microscopically and placing dilutions of the washings in agar. In addition, seeds from several infected samples were soaked in a clearing agent, pyridine, for 48 hours, washed, and soaked in an aqueous solution of propionic acid and carmine overnight.

The examination of the washings showed that no conidia or mycelial fragments of *A. imperfecta* were present and no colonies of this fungus developed on the agar in which drops of the washings had been placed. A microscopic examination of the alfalfa seed which had been cleared and stained showed that mycelium was growing in the surface of several of the seeds. This mycelium could not be identified definitely as that of *A. imperfecta*, but because its measurements were appropriate and because *A. imperfecta* was the commonest fungus among the isolates from the alfalfa seed, it is probable that the mycelium on the seed belonged to this fungus.

Effect of Seed Treatment

The effect of fungicides on the growth of *A. imperfecta* from naturally inoculated seed of alfalfa was studied in the laboratory by plating untreated and treated seed on potato dextrose agar in Petri dishes and incubating them for 10 days at 20° C. The fungicides which were used and the rates of application (percentage of weight of seed) were as follows: Ceresan 0.09, Arasan 0.25, Semesan 0.25, Spergon 0.25. The efficiency of these fungicides was measured by the suppression of growth of *A. imperfecta* from treated seeds as compared with untreated seeds. Daily examination of the Petri dishes showed that young colonies of fungi, most of which were identified later as *A. imperfecta*, were growing from untreated seeds in 15 to 20 hours, while growth of fungi from the treated seeds was suppressed entirely for 5 to 6 days and then it occurred on very few seeds. The alfalfa seed germinated well on the agar and seedlings developed rapidly but, in

TABLE 2.—THE EFFECT OF CHEMICAL TREATMENT ON THREE SAMPLES OF ALFALFA SEED NATURALLY INFECTED WITH *Ascochyta imperfecta*, PLATED ON AGAR IN PETRI DISHES

Treatment	Seeds yielding colonies of *A. imperfecta*	Classes of seedlings		
		Healthy	Infected	Dead
	%	%	%	%
Ceresan	0.3	90.7	5.3	4.0
Arasan	0.0	93.0	4.0	3.0
Semesan	0.7	97.0	2.0	1.0
Spergon	0.7	93.3	1.0	5.7
Check	23.3	41.3	14.4	44.3

the case of untreated seed, many of the seedlings became infected and many of them died. The results obtained in a representative test with three moderately infected samples are given in Table 2.

All of the fungicides were reasonably effective in inhibiting *A. imperfecta* and very few of the seedlings from treated seed were infected by the fungus. An average of 23.3 per cent of the untreated seed produced colonies of *A. imperfecta* and many of the seedlings were parasitized or killed by the parasite. Ceresan injured the germination of the alfalfa seed slightly, while Arasan, Semesan, and Spergon were slightly beneficial.

Inoculation of Alfalfa Seedlings

The effect of *A. imperfecta* on alfalfa seedlings was studied in greenhouse and laboratory experiments. In these experiments the seed was first surface sterilized with a solution of calcium hypochlorite, rinsed in water and then dipped in a suspension of spores of *A. imperfecta*. The inoculated seed was air dried at about 70° F. and was plated on moist filter paper in Petri dishes and sown in moist sand and soil in cardboard boxes. Notes on seedling growth and disease development were taken 10 days after planting.

During normal germination of alfalfa, the hypocotyl elongates rapidly and carries the seed coat upwards, clasping the cotyledons. The seed coat forms a shield for the cotyledons as they are forced by the hypocotyl through sand or soil but it also provides a source from which a parasitic fungus which is seed-borne may attack the cotyledons and the upper part of the hypocotyl. When growth of the seedling is rapid as in healthy seedlings, the cotyledons expand quickly, shed the old seed coat and function as the first leaves.

In the course of the inoculation experiments several distinct forms of injury by *A. imperfecta* on the alfalfa seedlings were observed. In the early stages of germination of some infected seeds, the young radicle was invaded by mycelium present in the seed coat and destroyed before it had grown more than a few millimetres. Most of the seeds germinated and the hypocotyl elongated, but the seed coat remained clasping the cotyledons. Examination of the apical portions of the seedlings in this case showed that the tissues of the hypocotyl and cotyledons had been penetrated by hyphae which grew from the seed coat. This infection usually killed the seedlings in 7 to 10 days (Figure 1A). This type of injury occurred more frequently in Petri dishes where the air was very humid than in boxes of sand or soil on greenhouse benches. A third form of seedling infection occurred in which the seed coat was shed by the cotyledons and remained clasping the base of the hypocotyl. In this case, hyphae grew from the seed coat and penetrated the hypocotyl causing decay of cortical tissues. This type of injury seldom killed the seedling (Figure 1B). The results of two experiments on inoculation of alfalfa seed with *A. imperfecta* are given in Table 3.

As shown in Table 3, the number of dead seedlings corresponded closely with the number infected at the apex of the hypocotyl and on the cotyledons. The reason for that close agreement is that the young plumule, which includes the growing point, lies between the cotyledons at the apex of the hypocotyl, and is affected by any extensive injury of the tissues in that area. Numerous examples of this were found when the infected tissues

TABLE 3.—THE EFFECT OF INOCULATING ALFALFA SEED WITH *Ascochyta imperfecta*

Medium	Series	Classes of seedlings			Dead seedlings*
		Healthy	Base of hypocotyl infected	Cotyledons and apex of hypocotyl infected	
		%	%	%	%
Soil	Inoculated	38	1	61	54
	Check	96	0	4	4
Sand	Inoculated	19	6	75	75
	Check	90	6	4	4
Filter paper	Inoculated	2	2	96	96
	Check	90	3	7	7

* Included in "Classes of seedlings".

at the apex of the hypocotyl were dissected and examined microscopically. A description of the condition of these tissues will be given in the next section.

The Fungus in Seedling Tissues

The mode and development of infection of seedling tissues by *A. imperfecta* was studied by means of microtome sections. Infected portions of 7-day-old seedlings were fixed in formalin acetic alcohol solution, processed in butyl alcohol, sectioned, stained with safranin and mounted in Canada balsam.

Microscopic examination of sections of the base of the hypocotyl where lesions occurred showed massing of mycelium on the surface of the hypocotyl and multiple invasion of the cortical tissues (Figure 2). Branching, septate mycelium was inter- and intracellular, and the affected tissues were in various stages of disintegration. The massing of the mycelium occurred on the walls of the inner cortical cells also, but less frequently than on the exterior walls. Individual strands penetrated almost to the vascular tissue in the centre of the hypocotyl. At certain locations, large rounded masses of mycelium were found imbedded in the cortical tissue, surrounded by denser layers which had the appearance of pycnidial walls (Figure 3). These structures may have been the rudiments of pycnidia.

Portions of diseased seedlings which had been killed by invasion of the apex of the hypocotyl and the cotyledons by mycelium present in the seed coat were examined in microtome sections. This examination showed that the tissues of these organs were ramified with mycelium and, in the case of the hypocotyl, had collapsed. The cotyledons had failed to expand and function as leaves. Infection had spread from the cotyledons to the young leaves of the plumule, and from there to the growing point. The presence of mycelium in the plumule was demonstrated by teasing out the tissues in a mixture of lactophenol and acid fuchsin. This mixture cleared the tissues and stained the mycelium.

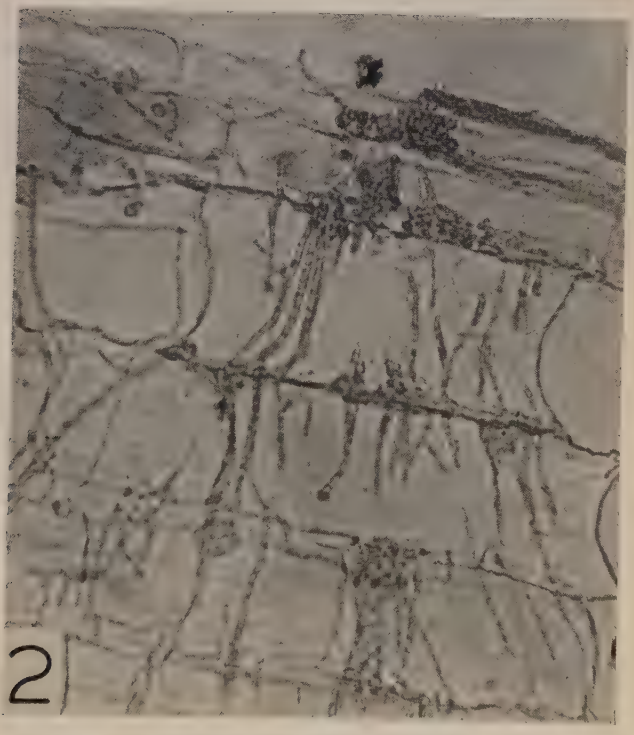

FIGURE 1. Seedlings of alfalfa infected with *A. imperfecta* showing (*a*) infected cotyledons and apical portion of the hypocotyl, and (*b*) infected basal portion of the hypocotyl. (X3).

FIGURE 2. Section of the basal portion of the hypocotyl of an alfalfa seedling showing cortical tissue invaded by *A. imperfecta*. (X320).

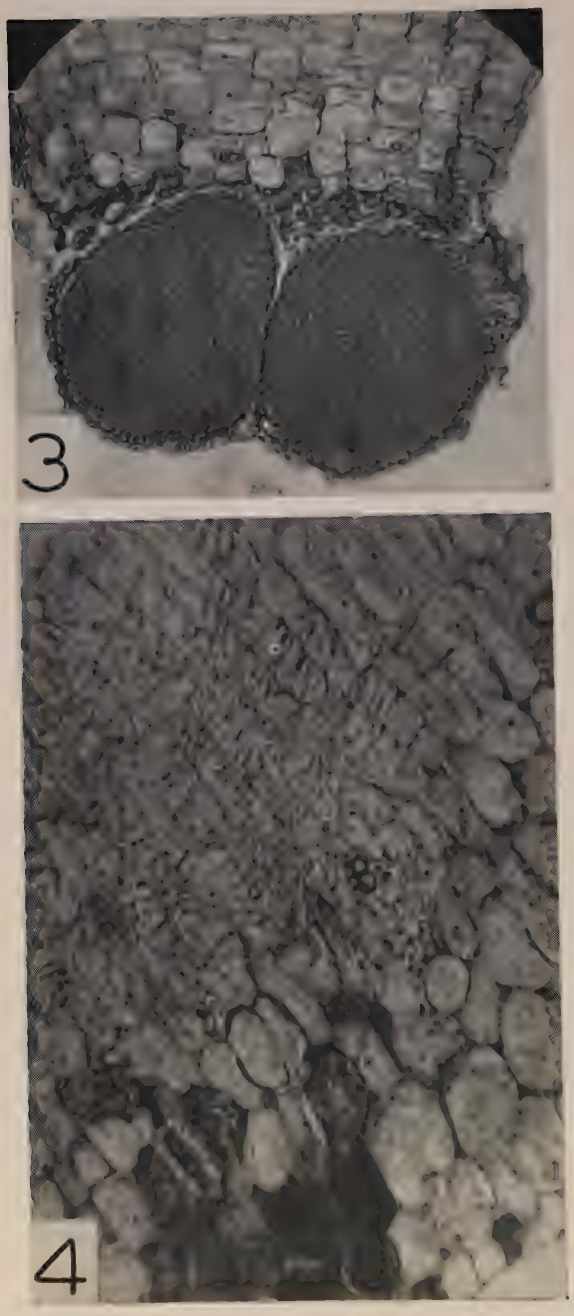

FIGURE 3. Mycelium of *A. imperfecta* massed in walled structures at the base of the hypocotyl of an infected alfalfa seedling. (X175).

FIGURE 4. Longitudinal section of the cotyledon of an alfalfa seedling showing mycelium of *A. imperfecta* in the tissues. (X325).

DISCUSSIONS AND CONCLUSIONS

The occurrence of *A. imperfecta* in seed samples from northern Saskatchewan was demonstrated in laboratory experiments. The fungus was obtained from 43 of the 58 samples which were tested and it occurred on 33 per cent of the seeds in a few of these samples when the seeds were plated on agar without surface sterilization. The fungus was not isolated by plating drops of the water in which the seed had been washed and no conidia were observed in the washings. When seeds from infected samples were cleared and stained, microscopic examination of these seeds showed that mycelium was established on the seed coat. This mycelium probably belonged to *A. imperfecta*.

It was shown in greenhouse and laboratory tests that *A. imperfecta* was pathogenic to alfalfa seedlings when spores of the fungus were carried on the seed and that under moist conditions it caused severe blighting of the seedlings. The importance of this form of carry-over and transmission of black stem is not known and is difficult to demonstrate in the absence of disease-free areas in the districts where alfalfa is grown extensively. Experimental evidence showed that seedling blight was severe where the atmosphere was moist. This condition exists in the form of heavy dews in many of the areas where alfalfa is grown and it probably would be favourable towards this type of injury. One instance is known of a newly-sown plot of alfalfa becoming infected with *A. imperfecta* in its first year of growth, apparently from seed-borne inoculum. A plot of alfalfa was sown at the experimental sub-station at Regina in 1951 with seed from northern Saskatchewan and the young alfalfa plants became infected that season. This station is situated over 200 miles from alfalfa-growing areas and alfalfa had never been grown on it previous to 1951. It seems likely that the infection on the seedlings developed from seed-borne inoculum.

The results of greenhouse and laboratory experiments showed that Ceresan, Semesan, Arasan and Spergon inhibited almost equally the growth of *A. imperfecta* from alfalfa seed, and that over 90 per cent of the seedlings developing from infected seed which had been treated were healthy compared with about 25 per cent from untreated seed. It would seem, therefore, that treatment of alfalfa seed with these fungicides, Arasan and Semesan particularly, could be recommended for the control of black stem in so far as it is seed-borne. Blighting of seedlings of alfalfa by *A. imperfecta* under field conditions is considered to be much less important than the damage done to young shoots and, later, to stems, leaves and pods.

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AN ELABORATION OF THE TOOTHPICK METHOD OF INOCULATING PLANTS[1]

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ABSTRACT

Quill-type, wooden toothpicks are boiled and rinsed in three changes of tap water. Tips of suitable length are placed, sharpened end up, in small glass vials; potato-dextrose broth is added until the tips are just covered; the vials are plugged, autoclaved, and inoculated in the usual manner and autoclaved for the requisite period of time. Retained in a ring around the inside wall of a suitable container by means of a cardboard "collar", the vials can be group-labelled and handled conveniently through all manipulations. In inoculating, mycelium-enveloped tips are inserted into awl-punctures in the stem or other parts, the protruding ends are cut off, and the wound is sealed with vaseline.

In recent studies of stem canker and of pod and stem blight of soybeans, caused by *Diaporthe* sp., and by *Diaporthe phaseolorum* (Cke. and Ell.) Sacc. var. *sojae* (Lehm.) Wehm., respectively, large numbers of plants had to be inoculated in order to evaluate the pathogenic capabilities of numerous isolates of both organisms. It became necessary to devise a method for inoculating plants rapidly and uniformly both in the field and in the greenhouse. The toothpick method as described by Young (2) in 1943, and later, with modifications, by Crall (1) in 1952, was considered for this purpose and tested. Further modifications were made and the new technique proved to be more advantageous for our purposes, especially in carrying out the inoculations in the field. The modified toothpick method is described below.

Quill-type, wooden toothpicks (Figures 1 and 2) are boiled and rinsed in three changes of tap water to get rid of possible toxic or other fungus-inhibiting agents. When the toothpicks are dry, tips of uniform length are quickly obtained by making use of a small board, about $6\frac{1}{2} \times 3\frac{1}{2} \times \frac{3}{4}$-in. through which have been drilled 5 rows of holes ($\frac{1}{4}$-in. diam.), 10 holes per row (Figure 1). With the longer side of the board facing the operator, toothpicks are placed from left to right in the holes of the vertical rows 1, 3, 5, 7, and 9. With a pair of "side cutters" (Figure 2), toothpick No. 1 (*lower left corner*) is cut off flush with the surface of the board, the tip being left in the hole. The remaining portion of the toothpick is reversed and placed, sharpened end down, in the companion hole to the right, and the cutting operation is repeated. By repeating the operation for each of the 25 toothpicks, it is possible to obtain in a few moments 50 tips of uniform length. The size and thickness of the board, and the number of holes in it, could be adapted to the number of tips required for any particular purpose.

The tips are now placed, sharpened end up, in small, straight-walled, glass vials of dimensions, 1.4×4.5 cm., a vial of this size accommodating 20 of the tips. Potato-dextrose broth is added until the tips are just

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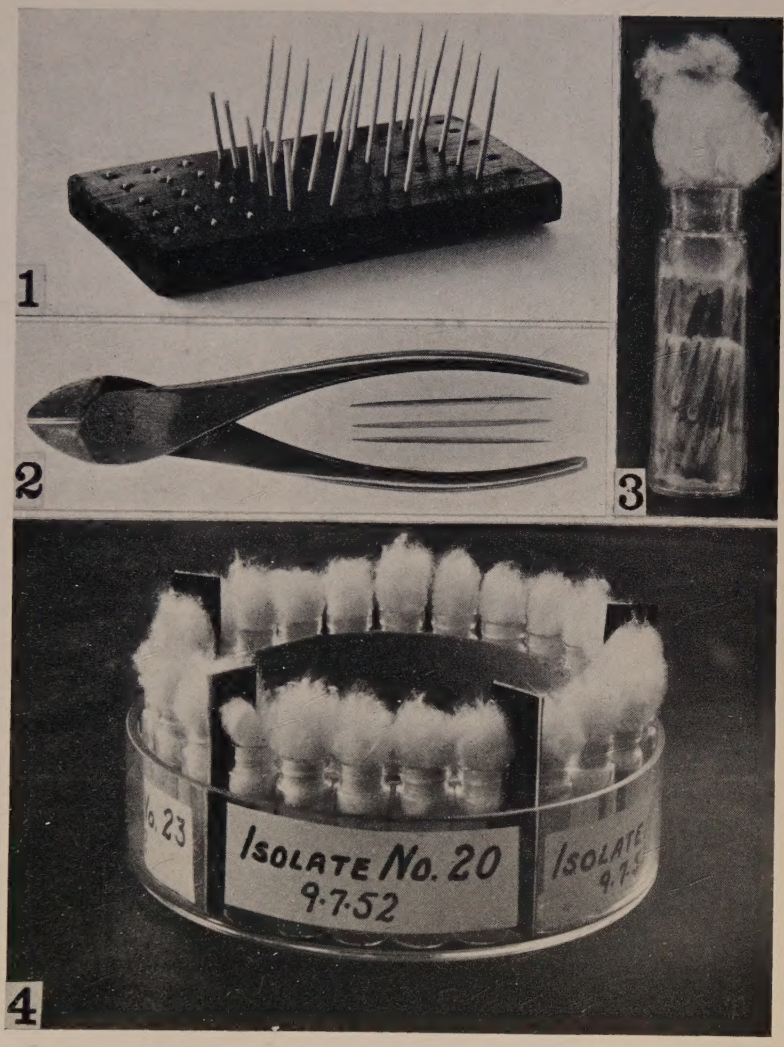

FIGURE 1. Board of required thickness with rows of holes in which toothpicks are placed for cutting.

FIGURE 2. "Side-cutters" used for cutting toothpicks.

FIGURE 3. Vial containing mycelium-enveloped toothpick tips.

FIGURE 4. Group-labelled vials retained in position in moist chamber by a cardboard "collar".

covered, and the vials, having been plugged with cotton in the usual manner, are autoclaved. Usually, during the latter process, the level of the liquid in the vials drops, and about $\frac{1}{4}$ in. of the tips may be left exposed. The vials are now inoculated with the organisms under test, and are then incubated in a suitable environment for the requisite period of time. The fungi used by the writer not only developed in and on the medium in the vials but also spread upwards on the tips enveloping each with mycelial growth (Figure 3).

For convenience and safety in handling throughout the various manipulations, the vials are placed in the lower (or the inverted upper) portion of a small moist chamber (diam. about 5 in.) and retained in a ring around its inside wall by means of a cardboard "collar" (Figure 4). In such an arrangement it is not necessary to label each vial individually; rather, a group of them, inoculated with the same organism, can be delimited and designated with a single label inserted between the vials and the wall of the container as shown in Figure 4.

The inoculating is done by first piercing the stem or other organ of the plant with a sharp awl and then inserting into the puncture a mycelium-enveloped toothpick tip. The protruding end of the latter is cut off flush with the surface of the stem by means of the "side cutters" (Figure 2) mentioned above. The puncture and the end of the toothpick-insert are then covered with a smear of vaseline. Without the latter, infection often failed to take place except in the case of the most aggressively parasitic of the isolates. Up to 400 inoculations per day were completed in the field by the above technique which is readily amenable to modification and adaptation to meet particular requirements.

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